ISSN 0377–9335

Vol. 27 December 2002 No. 4

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SHORT COMMUNICATIONS

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ENTOMON

ENTOMON is a quarterly journal of the Association for Advancement of Entomology issued in March, June, September and December, devoted to publication of research work on various aspects of insects and other arthropods.

Address MS and all editorial correspondence to Managing Editor, ENTOMON, Department of Zoology, University of Kerala, Kariavattom, Trivandrum 695581, India.

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ENTOMON 27(4): 339–346 (2002)
Article No. ent.27401

Searching behaviour and functional response of *Rhynocoris longifrons* (Stål) (Heteroptera: Reduviidae), a key predator of pod sucking bug, *Clavigralla gibbosa* Spinola

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ABSTRACT: Total searching time of fed *Rhynocoris longifrons* in the stem was 48.20 ± 16.15 minutes whereas its was reduced to 5.60 ± 5.12 minutes in the one day prey deprived *R. longifrons*. The fed predator seldom moved to the branches whereas the prey deprived ones were found in the branches in search of prey. The prey deprived *R. longifrons* were also present above and below the leaf surfaces. The number of prey killed (y) by the individual predator increased as the prey density (x) was increased from 1 prey/predator to 16 prey/predator. The predation rate showed a steep rise from 1 to 8 prey densities and it reached a plateau ($y = 1.008 + 0.258x$; $r = 0.989$) up to 16 prey densities. The maximum predation represented by K value was always found restricted to the higher prey density ($K = 4.8$) of *Clavigralla gibbosa* Spinola. The attack ratio decreased as the prey density was increased ($y = 0.755 - 0.0304x$; $r = -0.09610$). A negative correlation was obtained between prey density and the searching time of the predator at all prey densities ($y = 3.1612 - 0.2140x$; $r = -0.9597$). Again a negative correlation was obtained between prey density and the rate of discovery of *R. longifrons* at all prey densities ($y = 0.3329 - 0.0133x$; $r = -0.4674$) respectively.

KEYWORDS: biological control potential, *C. gibbosa*, searching behaviour, functional response, *R. longifrons*

INTRODUCTION

Insect pests are proved to be limiting factors in the ecosystem and in agriculture often causing total crop loss. Because of the high cost of protecting crops from insects pests with chemical insecticides, the increasing concern over residues in food and gradual

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depletion of natural resource, we have to switch over to integrated pest management (IPM) programme where the predators are an important constituent (Grundy *et al.*, 2000). Reduviid predators were identified as potential biocontrol agents against many insect pest and hence, its potential exists for an even greater role in IPM (Grundy and Maelzer, 2000). Ambrose (2000) reported that the multivoltine voracious harpactorine reduviid predators such as *Rhynocoris marginatus* (Fabricius), *R. kumarii* Ambrose and Livingstone, *R. fuscipes* (Fabricius) and *R. longifrons* (Stål) with higher fecundity and hatchability and ability to withstand climatic adversities could be effectively used in biological control programmes. *R. longifrons* is a polyphagous insect predator which predates on number of crop pests such as *Helicoverpa armigera* Hubner, *Exelastis atomosa* (Walsingham), *Clavigralla gibbosa* Spinola, *Nezara viridula* (L.) and *Riptortus pedestris* (Thunberg) (Claver and Ambrose, 2000). Its population dynamics, biology and predatory potential were studied by Kumar (1993) and he urged to exploit its biocontrol potential in crop pests management. Hence, the present study was conducted to assess the impact of prey deprivation on its searching behaviour and its functional response to the pod sucking bug *C. gibbosa* Spinola (Hemiptera: Coreidae).

MATERIALS AND METHODS

The predator *Rhynocoris longifrons* was collected from the Sunkankadai scrub jungle (77° 41′E and 8° 21′N) and Muppanthal (77° 31′E and 8° 22′N) scrub jungle near Kanyakumari, Tamil Nadu, India. They were maintained in round plastic containers (1500 ml capacity or 16 cm diameter ×7 cm height) in the Entomology Research Unit laboratory (28–34 °C temperature; 12–13 h photoperiod; 75–80% relative humidity) during 2000–2001. The laboratory mass-reared reduviids were used for the studies.

Searching behaviour

The foraging behaviour of *R. longifrons* was conducted in pigeonpea plants with pod bug *C. gibbosa*. Before starting the experiment the plant was shaken to keep the plant free of other insects. Then a pest was introduced and allowed for acclimatization. Ten minutes after introducing the pest, an adult *R. longiforns* was placed at the middle of the stem of the plant with a help of forceps. Its searching movements towards prey was recorded in the daily fed predators as reported by Claver and Ambrose (2001). The searching behaviour of the predator was watched continuously for 2 hrs. The experiment was repeated with predators prey deprived for one day. The searching movement in the stem, above the leaf surface, below the leaf surface, on the flower and on the land surface were directly observed along the predator's pathway to record the total distance travelled, the searching speed, the rate of turning and the angular change.

Functional response

The functional response experiments were conducted with 24 h prey deprived adult predators to the pigeonpea pest *C. gibbosa* at 1, 2, 4, 8 and 16 prey densities for

4 days. The prey was first introduced into the plant followed by the introduction of the predator. The plant with the prey and the predator was covered by a small mesh cage. The prey consumed or killed in 24 h was calculated. Killed prey was replaced daily during the experiment. Holling's 'disc' equation (1959) was applied to describe the functional response of *R. longifrons* to *C. gibbosa* as follows:

x = prey density; y = total number of prey killed in given period of time (T_t); y/x = the attack ratio; T_t = total time in days when prey was exposed to the predator; b = time spent for handling each prey by the predator, (T_t/K); a = rate of discovery per unit of searching time $[(y/x)T_s]$. The parameters b, K and a were directly measured in the present study. The handling time b was estimated as the time spent for pursuing, subduing, feeding and digesting each prey. The maximum predation was represented by K value and it was restricted to the higher density. Another parameter a at the rate of discovery was defined as the proportion of the prey attacked successfully by the predator per unit of searching time.

Discovery was instantaneous with little searching time being required. Although the parameter rate of discovery was theoretically infinite, the predator did spend some time in searching for the prey in lower prey density but no time at higher prey density.

Assuming that the predators efficiency is proportional to the prey density and to the time spent by the predator in searching prey (T_s) the expression of relationship is:

$$y = aT_s x \tag{1}$$

However, time available for searching is not constant. It is equivalent to the total time (T_t) minus the time spend for handling the prey (b). We presume that each prey requires a constant amount of time b for the consumption, then

$$T_s = T_t - by. \tag{2}$$

Substituting (2) in (1), Hollings 'disc' equation is

$$Y = a(T_t - by)x. \tag{3}$$

The regression analysis was made to determine the relationship between the prey density and the number of prey consumed, the searching time, the attack ratio and handling time (Daniel, 1987).

RESULTS AND DISCUSSION

Searching behaviour

Fed predators spent significantly less time than one day prey deprived predators. But on the contrary the fed predators spent 48.2 ± 16.15 minutes in the stem regions of the plant, whereas the one day prey deprived *R. longifrons* spent very less time on the plant (5.60 ± 5.12 min) (Table 1). Total distance travelled by *R. longifrons* increased as the days of prey deprivation was increased (Table 2). It recognized the prey from a distance and it attacked the moving prey. Starved for two day *R. longifrons* travelled significantly longer (111.52 ± 30.27 cm) distance than ones (49.27 ± 10.42) to find prey. Cisneros and Rosenheim (1998) stated that the starved predator *Zelus renardii*

TABLE 1. Searching behaviour of *R. longifrons* to *C. gibbosa* at pigeonpea plant shoot for 2 hours ($n = 5$; $\overline{X} \pm$ SD)

Condition Fed/prey deprived	Movements (in minutes)					
	Stem	Branch of stem	Above the leaf surface	Below the leaf surface	Flower of pod	Land surface
Fed	48.2 ± 16.13	0.0	0.0	0.0	0.0	0.0
One day prey deprived	5.6 ± 5.12	6.8 ± 9.54	21.2 ± 26.3	5.4 ± 7.79	0.0	0.0

Kolenati (Hemiptera: Reduviidae) spent most of its time in the upper plant strata and performed non-searching activities. The total distance travelled by the predator was greater at low prey density than that at higher prey density as reported by Wallin and Ekbom (1994) in a predatory carabid *Ptretostichus* sp. Moreover, Cisneros and Rosenheim (1998) and Awan (1990) emphasized that searching efficiency continued to rise as prey number became scarce. Hungry induced the predator's searching speed. For instance, searching speed for two day hunger *R. longifrons* (4.02 ± 1.32 cm/10 s) was significantly greater than fed *R. longifrons* (1.92 ± 0.36) (Table 2). Moreover, the predator's searching speed increased as the prey density was increased. The searching speed of the fed predator was significantly longer than that of one day deprived predator. The fed predators moved slowly from one place to another whereas the one-day prey deprived predator searched the stem, above the leaf surface and below the leaf surface very quickly. The fed predators were not found to move to the branches whereas the prey deprived ones were observed in the branches in search of food for 6.80 ± 9.54 minutes. The prey deprived *R. longifrons* were also present above and below the leaf surface for 21.2 ± 26.3 and 5.4 ± 7.79 minutes, respectively (Table 1). Again, higher rate of turning was observed in two day hunger *R. longifrons* (8.31 ± 2.54/10 s) than in fed *R. longifrons* (3.82 ± 0.13/10 s). Daily fed *R. longifrons* turned 4.52°/cm whereas two day prey deprived ones turned 8.38 ± 1.30°/cm) to find prey in the stem and leaf surfaces (Table 2). The searching speed of fed predators was significantly longer than one-day prey deprived predators, because fed predators moved slowly from one place to another. Claver and Ambrose (2001) stated that the angular change made by prey deprived predators was higher than that of fed ones because of lack of motivation in the well fed predators (Sandness and McMurtry, 1972; Blommers *et al.*, 1977). Similar responses were reported for predatory beetles and predatory anthocorid bugs at different prey densities and prey associates conditions (Chiverton, 1988; Coll *et al.*, 1997).

Functional response

The adult *R. longifrons* responded to increasing prey density of *C. gibbosa* by killing more number of prey than prey killed at lower prey densities, thus exhibiting type II functional response of Holling's (Table 3). The number of prey killed (y) by the individual predator increased as the prey density (x) was increased from

TABLE 2. Influence of hunger status on the searching behaviour of *R. longifrons* to *C. gibbosa* ($n = 7, \overline{X} \pm SD$)

Hunger status	Distance travelled in stem and leaf surface (cm)	Searching speed (cm/10 s)	Truning rate (10 sec)	Turning angle (degree/cm)
Fed	111.52 ± 30.25^{b}	1.92 ± 0.36^{a}	3.82 ± 0.13^{a}	4.52 ± 0.89^{a}
One day	78.61 ± 18.33^{ab}	3.14 ± 1.12^{b}	4.56 ± 0.72^{ab}	7.31 ± 2.26^{ab}
Two days	49.27 ± 10.4^{a}	4.02 ± 2.51^{b}	8.31 ± 2.51^{b}	8.38 ± 1.39^{b}

*Values followed by different alphabets are statistically significant by DMRT ($P = 0.05$).

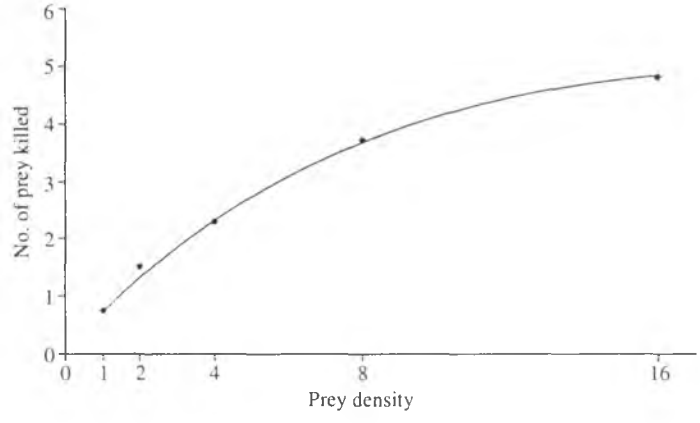

FIGURE 1. Functional response of *R. longifrons* to *C. gibbosa*.

1 prey/predator to 16 prey/predator. The predation rate showed a steep rise from 1 to 8 prey densities and a plateau was maintained at 16 prey density (Fig. 1) ($y = 1.008 + 0.258x$; $r = 0.989$). The maximum predation represented by K value was always found restricted to the higher prey density ($K = 4.8$) of *C. gibbosa*. According to Houck and Strauss (1985), functional response study could be used to infer basic mechanisms, underlying the interactions of predator-prey behaviour, to clarify co-evolutionary relationship and to enhance practical predictive power of biological control operations. The reduviid *R. longifrons* has positively responded to the increasing prey density by effectively suppressing the prey, *C. gibbosa*. When the prey density was increased, the number of prey killed by the individual predator also increased. Such enhanced predatory efficiency exhibited by predators in relation to prey density was reported by many authors in various predatory insects. The observation of Ambrose and Claver (1996) in *R. fuscipes* to pod sucking bug *Riptortus clavatus* Thunberg confirmed the present observation. Maximum number of prey was killed at 16 prey densities. The highest attack ratio was observed at the density of 1 prey/predator and the lowest attack ratio was found at the density of 16 prey/predator.

TABLE 3. Functional response of *R. longifrons* to *C. gibbosa* for 4 days ($n = 4$; $\overline{X} \pm$ SD)

Prey density (x)	Prey attacked (y)	Max. y (k)	Days per y (b) $= T_t/k$	Days all y's (by)	Days' searching $T_s = T_t - by$	Attack ratio (y/x)	Rate of discovery ($y/x/T_x = (a)$	Disc equation $Y = (T_t - by)x$
1	0.75 ± 0.43^a			0.62	3.38	0.75	0.22	
2	1.5 ± 0.71^b			1.25	2.75	0.75	0.27	$Y = 0.312$
4	2.3 ± 1.05^c	4.8	0.83	1.9	2.09	0.57	0.27	$(4 - 0.83y)x$
8	3.7 ± 1.48^{cd}			3.07	0.93	0.46	0.49	
16	4.8 ± 1.69^d			3.98	0.02	0.3	–	
							Mean = 0.312	

*Values followed by different alphabets (in the 2nd column) are statistically significant by DMRT ($P = 0.05$).

Hence, the attack ratio decreased as the prey density was increased ($y = 0.755 - 0.0304x$; $r = -0.9610$). The highest (0.75) and the lowest attack ratios (0.3) were observed at 1 prey/predator and 16 prey/predator, respectively (Table 3). It is presumed that the predator spent less time in searching activities which in turn might have caused a perceptive decline in the attack rate until the hunger was established. Hassell (1978) further attributed this response as the characteristic feature of type II functional response. The searching time decreased as the prey density was increased (Table 3). Hence, a negative correlation was obtained between the prey density and the searching time of the predator at all prey densities ($y = 3.1612 - 0.2140x$; $r = -0.9597$). The searching time, the interval between the consumption of a prey and the subsequent attack of the predator (Holling, 1959) decreased as the prey density was increased. This was due to the lesser searching time at higher prey density which in turn might have caused a perceptive decline in the attack rate. Moreover, probability of the predator's higher prey contact at higher prey density would have enhanced the searching ability per unit area. This observation was also confirmed by Ambrose *et al.* (2000) in *R. marginatus* to *C. gibbosa* and grasshopper *Hieroglyhus bannian* (F.) The rate of discovery of the prey decreased as the prey density was increased. Hence, a negative correlation was obtained between the prey density and the rate of discovery of *R. longifrons* at all prey densities ($y = 0.3329 - 0.0133x$; $r = -0.4674$).

The above findings on the effective searching behaviour and the positive functional response of *R. longifrons* suggest that it could be considered as a potential biocontrol agent in the IPM programme for *C. gibbosa*.

ACKNOWLEDGEMENTS

We must express our sincere thanks to Department of Science and Technology, New Delhi for the young Scientist Award (HR/OY/Z-13/98) to the senior author (MAC). Financial assistance from Tamil Nadu State Council of Science and Technology, Chennai (RJ/BSO 30031) to DPA is also gratefully acknowledged.

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(Received in July 2001; revised and accepted in May 2002)

ENTOMON 27(4): 347–353 (2002)
Article No. ent.27402

Effect of P-Soyatose on the developmental and economic characteristics of the silkworm *Bombyx mori* L.

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ABSTRACT: The effective (optimum) dose of the supplemented protein–P-Soyatose was identified to be 2 and 4 mg soybean protein for the female and male V instar larvae respectively and registered the maximum increase in the larval, cocoon and shell weights. The male larvae reached a maximum weight of 3.553 g with 0.991 g of silk gland, 1.998 g cocoon, 1.619 g pupa and 0.379 g shell. Similar increase in weight was also registered in the female larvae. The larval and economic characteristics of silkworm *Bombyx mori* decreased drastically after starvation. However, refeeding either with 4 mg soyprotein or mulberry for 15 min augmented the weight of silk glands and other economic characters highlighting the importance of nutrition on the development and rate of silk protein synthesis in *B. mori*.

KEYWORDS: *Bombyx mori*, P-Soyatose, starvation, refeeding, silk protein, cocoon

INTRODUCTION

Starvation induces an extension of larval duration (Srivastava *et al.*, 1983; Mathavan *et al.*, 1987), decrease in cocoon and shell weight, fecundity (Muthukrishnan *et al.*, 1978), decrease in the polysome levels, decrease in the transcription of RNA (Prudhomme and Couble, 1979).

Supplementary feeding in *Bombyx mori* L. results in multifarious effects which include increase in the concentration of haemolymph proteins (Nagata and Kobayashi, 1990; Krishnan *et al.*, 1995; Janarthanan *et al.*, 1999), larval body, silk gland and cocoon weight (Sarker *et al.*, 1995; Vanishree *et al.*, 1996). The aforementioned facts prove unequivocally the impact of nutrition on silkworm growth and the amount of silk synthesis. None of the study however reports the impact of nutrition on male and

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female silkworm larva. The present study on supplementation will definitely provide vital data to improve silk and seed production.

MATERIALS AND METHODS

Eggs of hybrid, between a multivoltine strain of *B. mori* (Local, Tamilnadu white) and bivoltine, NB_2D_4 were purchased from the local Government Grainage, Tiruchirappalli, India and maintained at a temperature of 27 ± 2° C and 75 ± 5% relative humidity. Larvae that hatched out were fed with freshly chopped tender leaves of mulberry MR_2 variety until third instar and were given coarse leaves until the end of fifth instar. The feeding regime adopted was 5 times a day. Fifth instar larvae cease feeding to begin cocoon spinning on day 7 and larva-pupal ecdysis occur on day 10.

The supplementary nutrient chosen for the study was hydrolyzed soyprotein, P-Soyatose (Warkem Co., Mumbai, India). It is an enzymatic digest of soyprotein, containing 8.8% total nitrogen. This plant protein hydrolysate is neutral in pH, soluble in water and free from pathogenic organisms. Hydrolyzed soyprotein is devoid of trypsin inhibitors and it is highly nutritive (Horie and Watanabe, 1983; Krishnan *et al.*, 1995; Vanishree *et al.*, 1996).

Freshly ecdysed fifth instar larvae were sexed and each sex was divided into 4 groups of 30 larvae each. While one of the groups in each sex served as control, fed only on mulberry leaves, the other 3 groups of males received 2, 4 and 6mg/larva/day respectively, while those of females received 0.5, 2 and 3.5 mg larva/day 20% aqueous solution of soyprotein respectively, before the first mulberry feed. Such supplementation was given from day 2 to 6 of fifth instar.

Since starvation and refeeding evoked similar response in both sexes, only the one group was used for this experiment (Fig. 1). Male larvae on day 4 of fifth instar were starved after the first mulberry feed. The period of starvation was calculated 3 h from the mulberry feed. Starvation was imposed for 18 h, after which either mulberry feeding for 15 min or soyprotein at the level of 4 mg was fed to the larvae.

In the supplemented groups, the paired silkglands were dissected out each day from day 1 of fifth instar and their wet weights were recorded. Silk glands from the starved group were collected at 21, 24 and 30 h of starvation and from the refed groups after 3, 6 and 12 h after refeeding corresponding to the timings of sample collection after starvation and compared with the silk gland weights on day 5 of control larvae.

RESULTS

In the present study, the effective dose of soyprotein required by the fifth instar larvae of *B. mori* was identified by taking larval weight and economic characters (cocoon, pupal and shell weight) as index.

The larval weight was the maximum in the group that received 4 mg soyprotein/larva/day and recorded 3.553 g ($P < 0.001$) while it declined as the levels of soyprotein raised. It was 3.393, 3.210 ($P < 0.01$) and 3.124 g in the groups that received 5, 6 and 7 mg soyprotein/larva/day respectively. A similar trend

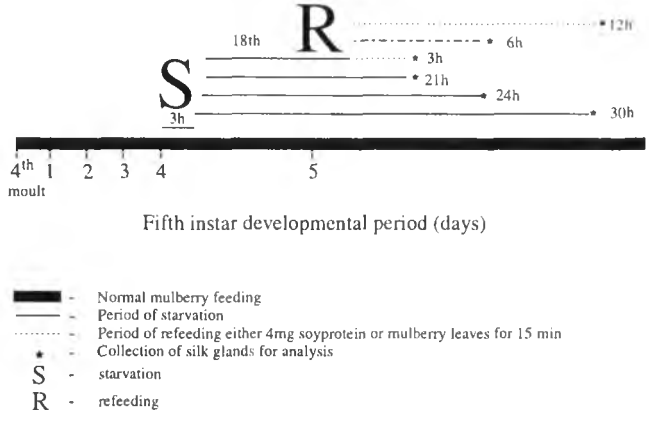

FIGURE 1. Diagrammatic representation of the time at which starvation was imposed duration of starvation, time when refeeding was given and time of sample collection.

was also observed in the females except for the larval weight, which was found to be higher for the females than that of the males. Weight of the larvae was higher in the soyprotein-supplemented groups and a biphasic dose-dependant pattern was observed. The highest larval weight of 1.372 g ($P < 0.05$) was observed in the group that received 2 mg soyprotein/larva/day which declined thereafter in the groups that received higher amounts of soyprotein.

The larval weights obtained by supplementation were fitted on a curvilinear regression using the equation $Y = a + bx + cx^2$ (Zar, 1999). The biphasic dose-dependant response of the larval weight with respect to the amounts of soyprotein received by the larvae is evident from Fig. 2(a). Two way analysis of variance of the data for both sexes, showed that the level of soyprotein received during fifth instar developmental period significantly (ANOVA- male–$F = 4543.67$, $P < 0.01$: Female–$F = 2278.051$, $P < 0.01$) influenced the larval weight.

The economic characters such as cocoon, pupal and shell weight of both the sexes of *B. mori* larvae that received soyprotein during fifth instar development, recorded higher values when compared to control. Although the maximum weight of cocoon and pupa were higher in females than males, it was the reverse with respect to the shell weight, implying a higher and stronger effect of soyprotein supplementation on the shell weights of male than female larvae. A comparison between the economic characters showed that the shell weight recorded the highest percentage increase in both sexes, implicating the effect of soyprotein supplementation on the silk producing apparatus, the silk glands.

The results obtained were fitted into a curvilinear regression using the equation $Y = a + bx + cx^2$ (Fig. 2(b)–(d)). The curvilinear regression of the cocoon, pupal and shell weight showed a biphasic dose-dependant response. All the three economic

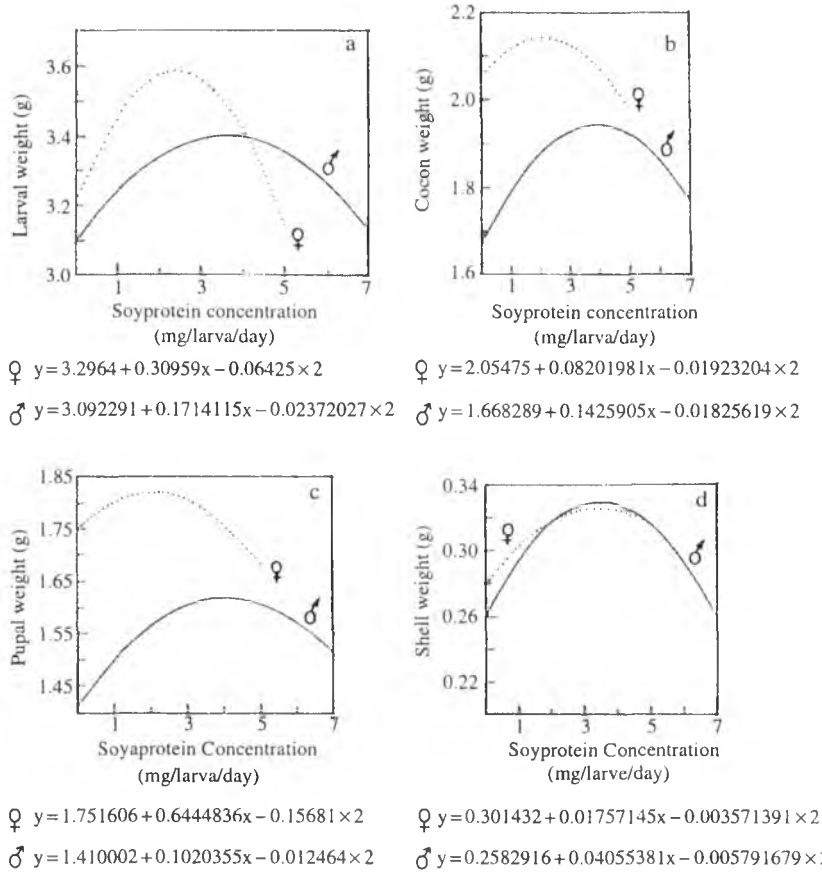

FIGURE 2. Effect of supplementing various concentrations of soyprotein on larval (day 6 of V instar) (a), cocoon (b), pupal (c), and shell weight (d) weight of male and female *Bombyx mori*.

parameters in both sexes were higher in the groups that received soyprotein during fifth instar development.

The silk gland weight monitored during fifth instar development showed clearly the effect of soyprotein on the silk producing apparatus of *B. mori*. The initial weight (wet weight) of a pair of silk glands from a single male larva on day 1 of fifth instar was 0.053 g. The weight increased in all the groups until the day of spinning. A similar trend was observed in the female larvae also, with the initial weight of silk glands on day 1 being 0.090 g, reaching a maximum of 0.891 g on day 6 in controls.

Contrary to supplementation, starvation resulted in a decrease in silk gland weight. Refeeding as little as 4 mg soyprotein could stimulate the effect of mulberry fed for 15 minutes. When only mulberry was refed to the larvae, the highest silk gland weight

was observed after 12 h of feeding, while in the soyprotein refed group, the highest silk gland weight was attained at 6 h and maintained at 12 h.

DISCUSSION

In the present study, fifth instar silkworm larvae were used for supplementation, as supplementation in early instar does not improve the cocoon quality and larval rearing. The total consumption during larval period of *B. mori*, over 80% are consumed during the final instar and the silk which is spun out finally as cocoon is that synthesized during the fifth instar. Prudhomme *et al.* (1985) also reported that silk produced in early instar is degraded during subsequent moults, hence supplementation in the earlier instars does not improve the cocoon production in addition to increasing the cost of rearing.

In the present study hydrolyzed soyprotein, devoid of trypsin inhibitors, increased the larval weight and economic characters of *B. mori*. The effective/optimal dose signifies fastest growth to the greatest size with highest fecundity (Dadd, 1970). Earlier studies also showed that nutritive proteins like soybean were known to promote growth and improve the economic characters of the silkworm (Ito, 1981; Krishnan *et al.*, 1995; Nirmala *et al.*, 1996; Janarthanan *et al.*, 1999). Supplementation with soybean protein resulted in better silkworm growth (Ito, 1981; Vanishree *et al.*, 1996) as it contained all the indispensable amino acids (Reinecke, 1985). With the increase in concentration of the supplement a decline in larval growth and increase in mortality were observed in the present study. Horie and Watanabe (1983) performed a comparative study by varying the concentration of soybean meal (artificial diet) from 20–60% and reported that growth of *B. mori* was maximum on the diet containing 30% soybean meal which declined as the concentration increased further. Krishnan *et al.* (1995) showed that the optimal concentration of hydrolyzed soyprotein needed by the fifth instar *B. mori* was 2% for more silk and seed production. Such a biphasic dose-dependant response had been observed not only in *B. mori* but also in other insects, *Teleogryllus commodus* (Burgess *et al.*, 1991), *Blatella germanica* (Cooper and Schal, 1992) and *Spodoptera fugiperda* (Whitford *et al.*, 1992).

In the present study, the optimum amount of soyprotein required for maximum growth and silk synthesis was different between male and female larvae. The results clearly indicated that the optimum dose required for the soyprotein was higher for males than females. However, the economic characters like larval, pupal and shell weights recorded the maximum in females than in males.

The present study revealed that female pupae showed higher weight than male irrespective of any experimental conditions. Tojo *et al.* (1980) reported that female larva accumulates more energy in the form of protein in silkworm *Bombyx mori* to supply energy for non-feeding pupal development and adult formation. Similar observations were also made in other insects such as *Manduca sexta* (Kramer *et al.*, 1980) and *Calliphora vicina* (Levenbook and Bauer, 1980). Since the female pupal weight is very well correlated with egg number in *Bombyx mori* (Jeyaswal *et al.*, 1991), heavier female moths by supplementation, can go for higher fecundity (eggs/female).

The present results, where the silk gland parameters registered an increase in the soybean refed groups earlier than those fed on mulberry, falls in line with the results of Bosquet (1983) on polysome levels. Mulberry being a complex diet, has to be digested completely before being absorbed for developmental activities, whereas the hydrolyzed soyprotein, being easily digestible, could have paved way for easy digestion, absorption and hence a quick resumption.

ACKNOWLEDGEMENTS

We thank the International Foundation for Science (IFS), Sweden, for financial grant [M.K. Grant No. B/2520-1], Council of Scientific and Industrial Research (CSIR) [Sanction No. 37/1054/00 EMR-II] and University Grants Commission (X.N).

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(Received in August 2001; revised and accepted in December 2001)

ENTOMON 27(4): 355–364 (2002)
Article No. ent.27403

Taxonomic accounts of new species of Drosophilidae (Insecta: Diptera) of Kumaon region, India

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ABSTRACT: Taxonomic account of one new species, *Hirtodrosophila hexaspina* and new distribution records of three other species, *Amiota biprotrusa*, *Drosophila bizonata* and *Hirtodrosophila quadrivittata* are given. Taxonomic relationship of the new species within the group is also discussed.

KEYWORDS: new species, Drosophilidae, Diptera, Kumaon, India

The Indian Drosophilid fauna has been studied extensively in recent years and most areas have been collected fairly extensively (Gupta, 1969, 1970, 1971, 1972; Gupta and Ray-Chaudhuri, 1970a,c; Gupta and Singh, 1977, 1979; Singh and Gupta, 1977a,b; Sing and Gupta, 1981; De and Gupta, 1996a,b; Reddy and Krishnamurthy, 1968, 1970).

The Kumaon region, a hilly area is located at an elevation of about 1938 metres (6000 feet) from the sea level on the northeast periphery of the state of Uttaranchal. It includes six border districts of the state viz. Nainital, Almora, Pithoragarh, Bageshwar, Champawat and Udham Singh Nagar. The area is characterized by having dense evergreen coniferous forest with medium to very steep slopes and extremely moist condition due to heavy rainfall. Despite its remarkable physiography, the state remained unexplored for its Drosophilid fauna until now. It is only recently some collection have been undertaken in this region which have yielded interesting results on Drosophilid fauna of this region (Singh and Bhatt, 1988; Singh and Negi, 1989, 1992, 1995; Singh and Dash, 1993, 1998; Singh and Fartyal, 2002; Singh *et al.*, 2000). This paper deals with the description of one new species and three new records of Drosophilidae from this region.

The flies were collected at several collection stations. Several different methods were employed to collect Drosophilidae, like (i) Sweeping through undergrowth or above debris on the forest floor; (ii) sweeping above rotting native fruits and artificially

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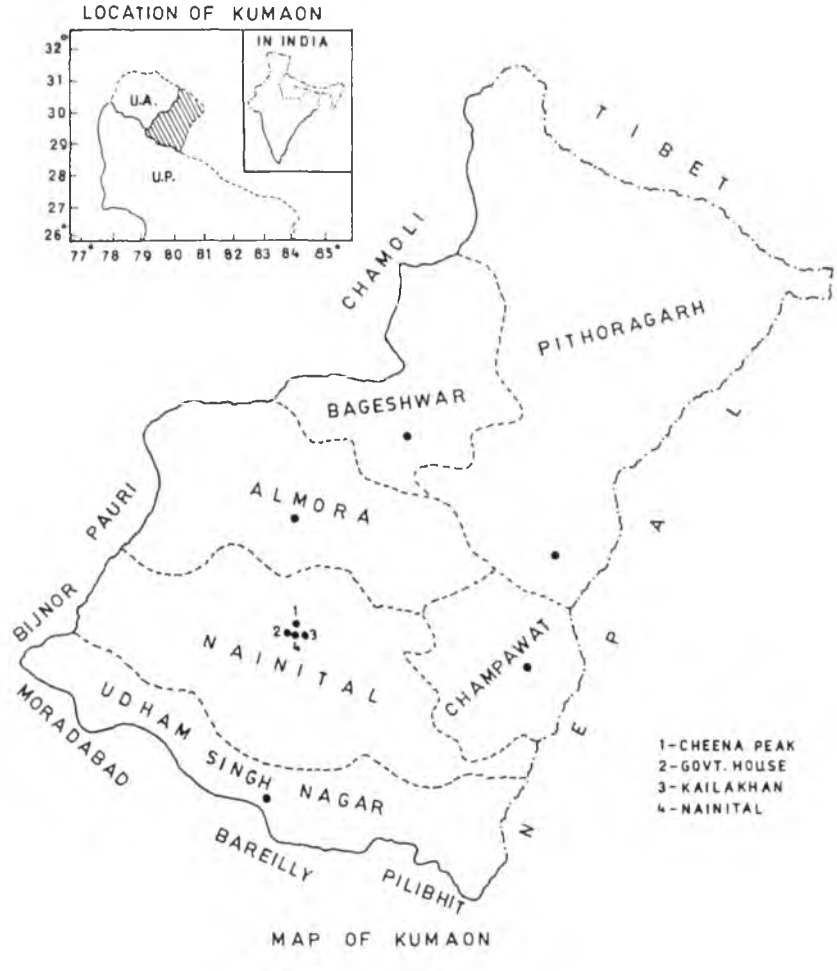

FIGURE 1. Map of Kumaon region showing collection localities.

yeasted fruit baits; and (iii) from fungi growing on decaying logs. Fig. 1 shows collection localities.

Genus *Hirtodrosophila* Duda

Hirtodrosophila Duda 1923, *Mus. Nat. Hungarici Ann.* **22**:41. Type species: *Drosophila latifrontata* Fronta-Pessoa, Taiwan.

The subgenus *Hirtodrosophila* Duda have recently been elevated to generic rank by A. D. Grimaldi, 1990, *Bull. Ann. Mus. Nat. Hist.* **197**: 1–139 (Bächli, 1998).

First flagellomere relatively large, with prolonged marginal hairs; subvibrissal setae small; Carina, if present, not broadened below; prescutellars absent; propleural setae absent; anterior and middle katepisternals usually very fine and small, anterior

reclinate orbital setae usually very fine. Over 100 species have been described in this genus and several species sub groups and species groups have been established.

***Hirtodrosophila hexaspina* sp. nov.**

Average length of the body: 3.80 mm (♂).

Head, ♂

Arista with about three upper and one lower branches in addition to terminal bifurcation. Antenna with pedicel and flagellomere grayish brown. Frons including the ocellar triangle dark brownish black. Orbitals in the ratio of 5 : 4 : 6. Facial carina light brown and flat, with one prominent apical and one small ventral setae. Vibrissa very long and prominent; subvibrissal setae minute. Gena yellowish brown and greatest width of gena 0.16 greatest dimeter of eye. Eyes bright red.

Thorax, ♂

Acrostichal setulae in about 8 regular rows. scutellum brown and scutellum uniformly brownish black. Basal scutellars slightly parallel and apical convergent; prescutellars absent. Mid katepisternals very fine and small; fore katepisternals moderate and hind very long and prominent. Sterno-index 0.67 legs pale yellow. Preapicals on hind tibia; apicals on mid tibia.

Wings, ♂ (Fig. 2D)

Veins brown. R-m and dm-cu crossveins clear. R_{2+3} slightly curved at costal margin. C_1 setulae two, unequal; C_3 fringe 0.33. Average wing vein indices: C-index 2.00; 4V-index 1.67; 4C-index 1.00; 5X-index 0.07. Halter stem and knob light brown.

Abdomen, ♂ (Fig. 2C)

Abdoiminal tergites brownish black. 2nd tergite medially interrupted with two lateral black spots; 3rd and 4th tergite with two lateral yellow spots 5th and 6th tergite black with two lateral yellow patches.

Periphallic organs (Fig. 2B)

Epandrium bow shaped with about four large setae in the upper part. Surstylus small, fused to epandrium with about 10–11 large setae. Cercus large, pubescent, oval and separated from epandrium with about 19–20 large setae.

Phallic organs (Fig. 2A)

Aedeagus large and pointed at the tip. Apodeme shorter than aedeagus: Parameters large, apically fused to hypandrium and serrated. Gonopod absent. Hypandrium somewhat quadrate, distally tapering with one pair of long paramedian margin and six dark brown teeth on the distal margin.

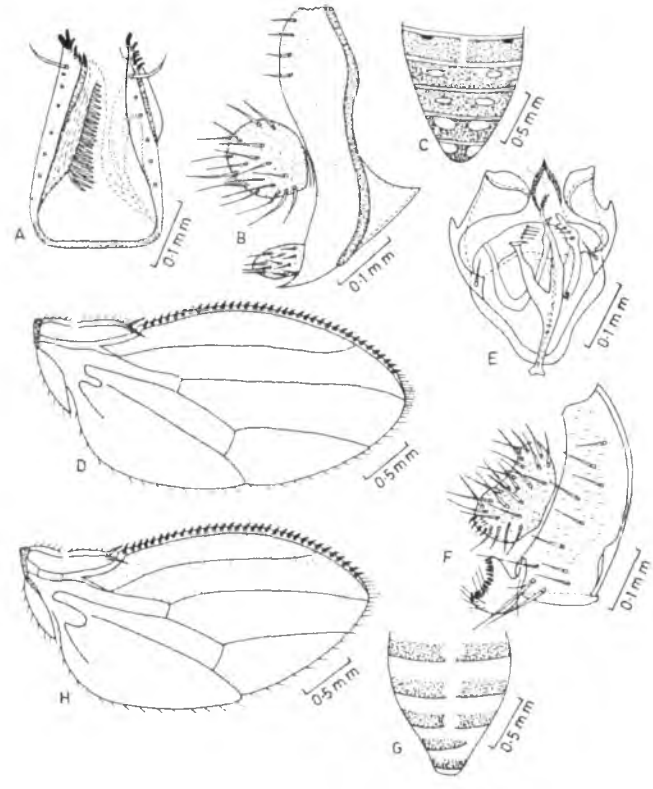

FIGURE 2. A–D: *Hirtodrosophila hexaspina* sp. No., A-phallic organs; B-periphallic organs; C-abdomen (♂); D-wing. E-H; *Hirtodrosophila quadrivittata*, E-phallic organs; F-periphallic organs; G-abdomen (♂); H-wing.

Holotype, ♂

India: Uttaranchal, Kumaon, Nainital district, Cheena peak, 10. IX. 1997. Cool. Fartyal an Singh by net sweeping on wild flowers. Deposited in the Department of Zoology, Kumaon University, Nainital.

Paratypes 2 ♂

Same data as holotype. Deposited in the Department of Zoology, Kumaon University, Nainital and the Institute of Low Temperature Science, Hokkaido University, Sapporo, Japan.

Distribution

Nainital, India.

Relationship

This species belongs to *hirtocornis* species group where it resembles *Drosophila hirtocornis* in the structure of periphallic organs but clearly differs from it in other structural details.

Key characters

Hirtodrosophila hexaspina sp. nov. can be differentiated form other species of the genus *Hirtodrosophila* by having R_{2+3} slightly curved at costal margin; 3rd, 4th, 5th and 6th abdominal tergites in male with two lateral yellow patches; surstylus small, fused to epandrium with about 10–11 large setae and hypandrium somewhat quadrate with 6 dark brown teeth on distal margin.

***Hirtodrosophila quadrivittata* Okada**

Hirtodrosophila quadrivittata Okada, 1956. 'Systematic study of Drosophilidae and Allied families of Japan'. Gihodo Co., Tokyo, Japan. pp 183.

Body length, ♂

Male 2.20 mm ($n = 1$).

Head, ♂

Arista with about 3 upper and 1 lower branches in addition to terminal bifurcation. Antenna with pedicel and flagellomere dark grayish brown. Frons including ocellar triangle dark brown. Orbital in the ratio of 5 : 4 : 5. Facial carina light brown. Palpus light grayish yellow, flattened, usually with 1 short and one prominent seta at the apex. Clypeus brownish yellow. Vibrissa prominent and subvibrissal setae minute. Gena yellow brown, greatest width of gena 0.15 the greatest diameter of eye. Eye dark red.

Thorax, ♂

Acrostichal setulae in about 8 regular rows. Scutum and scutellum dark brownish blak. Basal scutellar setae slightly divergent and apical crossing each other. Sterno-index 0.60. Hind katepisternals very long. Legs brown, preapicals present only on mid tibia; apicals on mind tibia. For 1st tarsomere as long as 2 succeeding tarsomeres together; mid and hind 1st tarsomere as long as 3 succeeding together.

Wing, ♂ (Fig. 2H)

Wing slightly fuscous. Veins grayish yellow; r-m and dm-cu crossveins clear. R_{2+3} slightly curved at the tip; R_{4+5} and M distally slightly convergent. C_1 setulae two equal; C_3 fringe 0.43. Average wing vein indices: C-index 2.00, 4V-index .89, 4C-index 1.11, 5X-index 1.78. Halter stem and knob light yellow.

Abdomen, ♂ (Fig. 2G)

Abdominal tergites pale yellow with dark brownish black bands. 2nd, 3rd and 4th tergites medially widely interrupted; 5th tergite slightly incomplete on the side and 6th tergite completely black.

Periphallic organ (Fig. 2F)

Epandrium brown, pubescent, narrow above and broad below with about 8–9 short and 2 long setae on the lateral margin. Surstylus fused with the epandrium with about 11–12 prensisetae arranged on outer concave row and about 7–8 short setae. Cercus triangular, separated from epandrium, pubescent with about 21–23 long setae and 6–7 short black prensisetae.

Phallic organs (Fig. 2E)

Aedeagus pointed at the tip and swollen of the base. Parameters large, curved and more or less triangular with about 3–4 sensilla. Gonopod long slender, gently curved outward and posteriorly fused to each other. Aedeagal apodeme shorter than aedeagus. Hypandrium triangular with 1 short paramedian spine.

Specimens examined

India: Uttaranchal, Nainital district, Govt. House, 2, ♂, 18. VII, 1999. Coll. Fartyal and Singh.

Distribution

Japan, Korea, China, Russia (Far east), India (new record).

***Amiota biprotrusa* Chen and Toda**

Amiota biprotrusa Chen and Toda, 1998, *Entomological Science*, **1(3)**: 403–407.
Body length, ♂ Male 5.00 mm. ($n = 2$).

Head, ♂

Arista with 6 upper and 3 lower branches in addition to terminal bifurcation. Antenna with pedicel dark brown and flagellomere brown. Frons including the ocellar triangle yellow brown. Orbitals in the ratio of 11 : 10 : 11. Palups somewhat triangular, yellowish brown with one prominent subapical and 2–3 lateral setae. Clypeus orange yellow. Facial carina light brown. Gena light brown and greatest width of gena 0.2 greatest diameter of eye. Eye red.

Thorax ♂

Acrostichal setulae in about 8 regular rows. Scutum brownish black and scutellum brown. Basal scutellar setae divergent and apicals convergent and crossing each other. Sterno-index 0.87. Legs yellow, tibiae with 3 dark brown rings Preapicals on all three tibiae and apical on 1st and 2nd tibiae only.

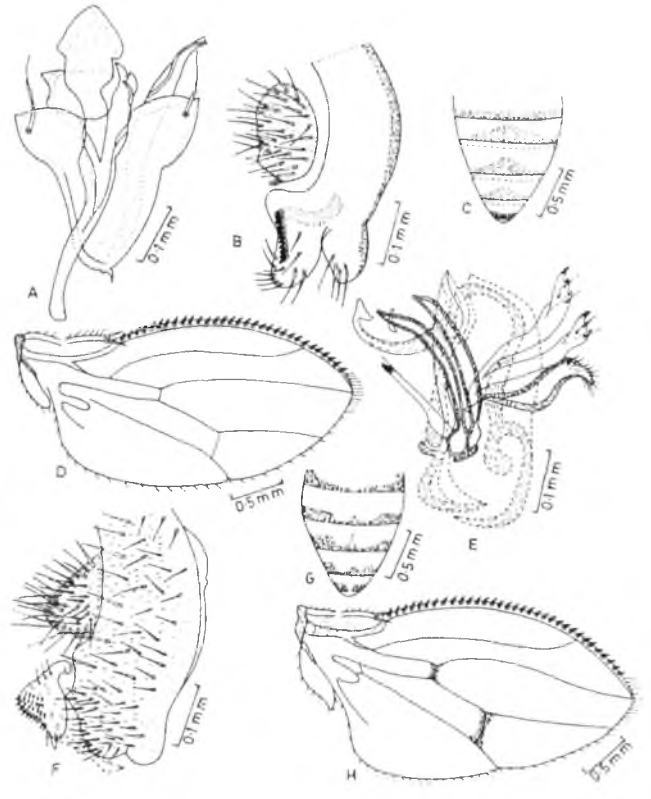

FIGURE 3. A–D: *Drosophila bizonata* A-phallic organs; B-periphallic organs; C-abdomen (♂); D-wing. E–H; *Amiota biprotrusa*, E-phallic organs; F-periphallic organs; G-abdomen (♂); H-wing.

Wing, ♂ (Fig. 3H)

Hyaline. Veins dark grayish brown, r-m and dm-cu crossveins clouded. C_1 setulae two, equal; C_3 fringe 0.69. Average wing vein indices; C-index 2.40; 4V-index 2.85; 4C-index 1.54; 5X-index 0.70. Halter stem and knob light brown.

Abdomen, ♂ (Fig. 3G)

Abdominal tergites yellow with black abdominal bands. 2nd abdominal tergite with black abdominal band interrupated in the middle, 3rd, 4th and 5th abdominal bands projected in the middle and 6th abdominal band interrupted in the middle.

Periphallic organs (Fig. 3F)

Epandrium broad above and below, highly pubescent with numerous setae. Surstylus triangular, separated from epandrium with about 25–26 prensisetae. Cercus triangular, pubescent and separated from the epandrium with about 35–40 long setae.

Phallic organs (Fig. 3E)

Aedeagus long and pointed at the tip with numerous sensilla. Parameters large and leaf like, serrated at the tip with 3 sensilla. Gonopods fused forming large median lobe. Aedeagal apodeme very short. Hypandrium narrow, curved and pubescent.

Specimens examined

India: Uttaranchal, Kumaon, Nainital district, Government House, Nainital, 2 ♂, 18. VII. 1999, Coll. Fartyal and Singh.

Distribution

China, Myanmar, India (new record).

***Drosophila bizonata* Kikkawa and Peng**

Drosophila bizonata Kikkawa and Peng, 1938, *Jap. J. Zool.*, 7: 532: *Drosophila (Drosophila) bizonata* Wheeler, 1949, Univ. Texas Publ., (4920): 190; Okada, 1955, Kontyu, Tokyo, 23: 98; 1956; Syst. Study Droso., 135.

Body length, ♂

Male 2.40 mm ($n = 2$).

Head, ♂

Arista with about 6 upper and 2 lower branches in addition to terminal bifurcation. Antenna with pedicel and flagellomere light brown. Frons including the ocellar triangle brown. Orbitals in the ratio of 4 : 6 : 5. Facial carina dark brown. Palpus yellow, with one apical and three ventral setae. Vibrissa prominent and subvibrissal setae half the length of vibrissa. Gena dark brown, greatest width of gena 0.16 the greatest diameter of eye. Eye dark red.

Thorax, ♂

Acrostichal setulae in about 6 regular rows. Scutum and scutellum dark brown. Apical scutellar setae upright and basal parallel; apical scutellars as long as basal, Sterno-index 0.6. Legs yellow; preapicals on all tibiae; apicals on mid tibiae.

Wings, ♂ (Fig. 3D)

Clear. Crossveins r-m and dm-cu clear. C_1 setulae two, equal; C_3 fringe 0.29. Average wing vein indices: C-index 3.00; 4V-index 1.83; 4C-index 0.83; 5X-index 1.78. Halter stem and knob light brown.

Abdomen, ♂ (Fig. 3C)

Abdominal tergites yellow with dark apical bands. 1st and 2nd abdominal bands complete; 3rd, 4th and 5th abdominal bands interrupted in the middle; 6th abdominal tergite completely black.

Periphallic organs (Fig. 3B)

Epandrium broad above and below; upper portion bare and lower portion with about four setae. Surstylus large and fused with epandrium with about 11–12 prensisetae arranged in a straight row and 10–11 long setae on caudo ventral margin. Cercus large, pubescent, separated from epandrium with about 27–28 long setae.

Phallic organs (Fig. 3A)

Aedeagus long and arrow shaped. Parameres large and leaf like, ventrally fused with hypandrium. Gonopod fused, horizontally flattened forming broad distal bow. Hypandrial apodeme triangular and notched. Apodeme longer than aedeagus. Hypandrium oval, Caudal margin with a pair of long paramedian spines.

Specimens examined

India: Uttaranchal, Kumaon, Nainital district, Kailakhan, 2♂, 27. VII. 1998. Coll. Fartyal and Singh by sweeping.

Distribution

Japan, Korea, Ryukyu's Nepal, Myanmar, Pyin Oo Lwin, Mandalay, Rangoon, India (new record).

ACKNOWLEDGEMENT

The authors are indebted to Dr. M. Toda, Institute of Low Temperature Science, Hokkaido University, Sapporo, Japan for confirming the identification of specimens. Thanks are also due to Council of Science and Technology, U.P. for providing financial assistance for present work.

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(Received in March 2001; revised and accepted in April 2002)

ENTOMON 27(4): 365–373 (2002)
Article No. ent.27404

Studies on screening and mechanism of resistance against the shootwebber *Antigastra catalunalis* (Duponchel)

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ABSTRACT: A methodology for screening Sesame accessions against the shootwebber in the field as well as under laboratory condition was evolved and ratings for field and caged conditions were standardized. Precise screening methodology in the early stage of the plant was formulated to take up large scale screening in short time. Out of 877 entries screened under field condition in hot spot area only three wild species namely *S. alatum*, *S. laciniatum* and *S. prostratum* exhibited high resistance,about 18 lines were found to be less damaged and scored resistance category. Out of 157 lines screened under caged conditions, only four entries i.e. Si 1115, ES 22,Si 250 and *S. alatum* recorded highly resistant score. Studies on the mechanism of resistance on the selected entries showed that *S. alatum* and Si 1115, non-preferred for oviposition, were found to have very few (Si 1115) or without any trichomes (*S. alatum*) when compared to TMV 3 having 13.5 trichomes/microscopic field at 450*x* magnification. The studies on the antibiosis mechanism on selected entries showed that *S. alatum*, ES 22 and si 250 adversely affected the larval period, larval weight and larval length. Growth index was high and reached maximum in the susceptible TMV 3 (7.13) when compared to *S. alatum*, ES 22 and Si 250 which recorded 1.55, 3.04 and 3.00 as growth index, respectively.

KEYWORDS: sesame shootwebber, screening, methodology, mechanism, resistance

INTRODUCTION

In the tropics and subtropics of the world, the sesame leafroller or Sim sim web worm or *til* leaf and pod borer *Antigastra catalaunalis* (Duponchel) (Pyralidae: Lepidoptera) is the most important pest of sesame (Weis, 1983). One of the difficulties in its control through insecticides is the concealed feeding behaviour of the insect (Singh *et al.*, 1980), which emphasize other management practices. Evolving of pest resistant variety will provide great interest to farmers as it involves no extra input. During this

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study, efforts were made to standardize a screening methodology both under laboratory and field condition and the mechanism of resistance was also studied.

MATERIALS AND METHODS

Field screening methodology

Eight hundred seventy seven germplasm entries of sesame were raised under replicated condition in 3 m rows with a spacing of 10 cm between plants and 30 cm between rows and uniform population was maintained by gap filling. All package or practices were followed as recommended in the Tamil Nadu Agricultural University crop production guide except plant protection. The entries were screened against the shoot webber based on the intensity of the infestation. Observation was made on 5 selected plants/entry at random for leaf, flower bud and pod damage. The following methodology was followed.

Leaf damage

Leaf damage for the shoot webber was recorded on 25, 40 and 60 DAG and per cent leaf damage was worked out based on the number of leaf damaged by the shoot webber and the total number of leaves during different stages and the mean per cent damage was arrived at.

Flower bud damage

The per cent of buds damaged by the caterpillar by observing the total and affected flowers on 45 and 60 DAG were calculated and mean percentage damage was worked out.

Pod damage

Number of pods damaged by the shoot webber was assessed and per cent pod damage was worked out.

Damage assessed on different plant parts at various stages was converted to 1 to 9 score by referring score chart. Score chart was formulated based on intensity of damage. As the damage on reproductive parts like flower and pods is reflected more than the leaf on yield, least pod, lesser flower and more leaf damage were equated to a particular score and the same is presented in the Table 1. After arriving at the cumulative score based on per cent damage on leaf, flower and pod for a particular entry, grad (1–9) was allotted by referring grade chart (Table 1) which was standardised based on the principles of standard evaluation technique followed for screening insect resistance in rise (Heinrichs *et al.*, 1985). Resistance level ranging from highly resistant to highly susceptible was estimated from the grade.

Procedure to evaluate the resistance level of hypothetical entry having 8, 12 and 1 per cent leaf, flower and pod damage is described below.

Step 1: By referring score chart (Table 1) for leaf (8%), flower (12%) and pod (1%) damage, the score 1, 5 and 1 are given.

TABLE 1. Methodology for scoring genotypes for shootwebber resistance (Field)

Score chart			
Per cent damage			
Leaf	Flower buds	Pod	Score
0–10	0–5	0–2	1
>10–20	>5–10	>2–4	3
>20–30	>10–15	>4–6	5
>30–40	>15–20	>6–8	7
>40	>20	>8	9

Grade chart		
Score	Grade	Mechanism
0–1	1	Highly resistant (HR)
>1–2	3	Resistant (R)
>2–3	5	Moderately resistant (MR)
>3–5	7	Susceptible (S)
>5–9	9	Highly susceptible (HS)

Step 2: Cumulative score for the damages is calculated i.e. $1 + 5 + 1 = 7$ and mean cumulative score is arrived at $7/3 = 2.3$.

Step 3: Mean cumulative score 2.3 is referred to grade chart which falls in the group between > 2 and 3 referring grade 5.

Step 4: Grade 5 is read against the resistance level which represents the category Moderately Resistant (MR).

Screening under laboratory

Based on the experience with the damage potential of the insect, visual grading system was fixed to screen sesame germplasms under laboratory condition to identify or/and confirm the field screened resistant genotypes in the early stage itself to accelerate the large scale resistance screening programme with the limited time with the same climatic conditions. Among small plastic cups (5 cm dia ×6 cm ht) or plastic trays, (10 × 15 cm) small tubular mud pots (10 cm dia ×15 cm ht) or nursery polythene bags (8 cm dia ×15 cm ht) used to grow the plants, the plants established well only in nursery bags and the same were used for sowing test entries. Sesame seeds were sown in nursery bags, kept inside the screening cage with three replication. Two different types of screening cages were tried for germplasm screening both with the dimension of 1 × 1 × 1 m. First type was made up of iron rods, covered with mosquito net all around and the other one with open bottom having wire mesh on four sides (0.5 × 0.5 × 0.5 mm) and the top of the cage was covered with a glass plate for easy penetration of light and to protect from rain. The former type cages were used in summer while later was used during rainy seasons. The seedlings were thinned at the rate of two/bag after 4 DAG and finally one/bag was maintained after 8 DAG.

TABLE 2. Grade chart for seedling screening

Extent of damage	Grade	Category
Partial loss of chlorophyll of one or two leaves or no damage at all	1	Highly resistant
Partial folding and loss of chlorophyll of one or more leaves of most plants	3	Resistant
Folding of four or more leaves and feeding or about half of the plants damaged or dead	5	Susceptible
Most of the leaves folded and damaged or all plants dead	7	Highly susceptible

When the plants were about 15 days old, 10 pairs of adults were released to screen about 50 lines. Sugar solution 10 per cent dipped in cotton swab was provided as food. Fifteen days after adult release entries were graded based on the intensity of leaf damage and grouped into different resistance level (Table 2).

Mechanism of resistance

Ovipositional non-preference

Sesame lines exhibiting resistance/susceptibility in the screening programmes were tested for ovipositional preference to *A. catalaunalis* in the laboratory under choice and no choice conditions with three replications. For ovipositional choice experiment individual germplasm lines were sown and maintained in the nursery polythene bags under screening cages (50 lines/cage) as done for screening programme. When the plants were two weeks old, about 10 pairs of adult insects were released into the cage through the doors provided in the cage. Cotton swabs dipped in 10 per cent honey solution were placed in cages as food. Eggs were counted consecutively for five days on each line and the mean number of eggs laid were calculated.

For no-choice experiment small wirenet cages (15 × 8 × 8 cm) were used to cover the potted single sesame plants. A pair of mated adult was released inside each cage and the moths were replaced if any death occurs within five days and the eggs laid were recorded consequently for 5 days.

Antibiosis mechanism

Individual sesame lines were sown in tubular mud pots. One week after emergence of plants newly emerged larvae from laboratory culture were transferred by moist camel hair brush (5/plant) and covered with cylindrical polyethylene transparent film cages. Accessions were replicated 10 times. The observations on larval and pupal development and weight were recorded from 10 plants from the date of releasing the larvae until pupation. Growth index was calculated by dividing the percentage of pupation with average larval period.

Trichome density

Lines exhibiting resistance were examined for trichome density and compared with susceptible lines. On 21st day after seedling emergence, second leaf from five randomly selected plants were sampled in each line. Standard procedures for clearing of the leaves for microscopic study was adopted for the observation of leaf trichome density as described by Maiti *et al.* (1980)

Leaf samples cut into segments of about 1–2 cm^2 were heated in 20 cc of water in small glass vials for 15 minutes in an incubator at 85 °C. The water was poured off and 20 cc of 96 per cent ethyl alcohol was added and the boiling procedure repeated to completely remove the chlorophyll from the leaf. The alcohol was again poured off ad 20 cc of concentrated (90%) lactic acid was added, the vials were stoppered and heated again at 85 °C until the leaf segments cleared (approximately 15 minutes). The vials were cooled and stored for observations.

To observe the trichomes, leaf segments were taken from the stored vials and mounted on the clean slides using a drop of lactic medium and trichomes were observed under a microscope at 450× magnification. Trichome number per randomly selected microscopic field was recorded.

RESULTS AND DISCUSSION

As the shoot webber damages almost all the parts of the sesame, a screening methodology was developed based on the damage potential of the pest on different parts of the plants. In general, the damage on reproductive parts is reflected more on yield rather than through the leaf. Hence, due weightage was given for the damage to flower buds and pods than to leaves.

Least pod and lesser flower per cent damage was equated to a particular grade as the pod damage directly reduce the yield, followed by flower damage. Vasiler and Bacnobe (1935) mentioned that though the larvae fed all the plant parts the damage was more by destroying buds, flowers, seeds and capsules. Single larva could destroy 2 to 3 plants in about a week and 4 to 6 flowers (Menon *et al.*, 1960). Sood *et al.* (1982) grouped the level of resistance into four categories based on per cent damage as resistant (0 to 5%), moderately resistant (6 to 10%), susceptible (11 to 20%) and highly susceptible (>20% damage). Hence, the maximum grade 9 to represent high susceptibility was given when the per cent damage was more than 40, 20 and 8 as against the minimum grade 1 indicating high level resistance for 0–10, 0–5 and 0–2 per cent in leaf, flower and pod, respectively.

Out of 877 lines screened under field conditions, only the three wild species tested (*S. alatum, S. laciniatum* and *S. prostratum*) exhibited high resistance scoring grade 1; 18 lines under resistant, 88 under moderately resistant; 409 under susceptible and the remaining 359 under highly susceptible category.

When the infestation takes place at a very early stage, the plants die without producing any branch or shoot (Menon *et al.*, 1960). Jakhmola and Yadav (1974) encountered difficulties in screening due to population fluctuation which can be

TABLE 3. Ovipositional preference of *Antigastra catalaunalis* on sesame accessions (Mean of three replications)

Accession	Oviposition (No. of eggs/plant (Mean ± S.D))	
	Choice condition	No choice condition
Es 22	12.7 ± 0.82	15.8 ± 2.10
Si 3315/5	11.8 ± 0.63	20.4 ± 3.10
Si 264	11.1 ± 1.28	15.7 ± 0.72
Si 1728	5.5 ± 0.75	12.5 ± 1.75
Si 3225	13.9 ± 1.91	23.2 ± 1.02
Si 1115	0.7 ± 0.24	3.1 ± 2.02
Si 250	9.0 ± 0.31	14.25 ± 1.21
Si 3170	11.2 ± 1.51	22.2 ± 2.84
Si 1/s	9.0 ± 0.31	14.5 ± 1.21
Is 304	10.2 ± 2.00	12.2 ± 2.20
Si 138	12.1 ± 0.78	14.5 ± 1.85
Si 3315/11	14.3 ± 2.71	19.2 ± 1.72
Si 2678	9.5 ± 0.44	14.5 ± 2.01
ES 12	11.2 ± 0.79	21.5 ± 2.12
Si 0022	13.5 ± 1.75	17.2 ± 1.91
Si 1050	7.9 ± 2.90	13.4 ± 1.53
Si 2785	9.8 ± 2.12	17.5 ± 0.83
Si 0020	12.7 ± 1.08	19.2 ± 1.21
Si 1523	13.5 ± 2.15	20.4 ± 2.21
Si 1528	11.5 ± 0.35	14.7 ± 1.04
Si 1582	9.7 ± 1.18	14.3 ± 3.01
Si 1780	11.5 ± 2.10	20.2 ± 1.92
Si 0126	12.0 ± 3.01	18.4 ± 2.71
RJS 51	12.5 ± 0.89	19.5 ± 1.05
IS 304	12.0 ± 0.95	20.2 ± 2.82
Pudukottai 6	10.3 ± 6.71	16.4 ± 1.05
TMV 3	15.4 ± 3.7	19.1 ± 1.51
TMV 4	14.5 ± 4.91	21.2 ± 2.32
Co 1	13.2 ± 8.66	18.9 ± 2.18
S. alatum	0.0 ± 0.00	0.5 ± 0.21

resolved by screening under laboratory conditions using mass reared insects. Seedling screening at 20 DAG helps in large scale screening to avoid the highly susceptible lines in the beginning of the experiment and also to confirm field evaluated lines. Hence, an accurate seedling screening methodology was developed based on the intensity of leaf damage for grouping the lines from highly resistant to highly susceptible category.

A total of 157 lines tested under caged condition was grouped, four in highly resistant, 6 in resistant, 104 in susceptible and 43 in highly susceptible category. Some of the lines N 66–250, N 66–276, Tc 289, etc.) found as resistant under field condition when subjected to caged screening fell under susceptible category. The reason might be evasion of insect infestation to the susceptible varieties under the field condition.

TABLE 4. Larval and pupal development of *Antigastra catalaunalis* on different sesame accessions (Mean of ten replications)

Accession	Larval period (days)	Larval weight (mg)	Larval length (mm)	Pupation (%)	Growth index
Es 22	11.5	15.5	9.5	35.0	3.04
Si 3315/5	9.2	23.0	13.7	61.7	6.70
Si 264	9.5	24.5	12.5	60.5	6.36
Si 1728	9.0	28.0	13.5	72.0	8.0
Si 3225	9.2	24.0	14.0	55.0	5.97
Si 1115	9.5	24.5	13.0	54.4	5.72
Si 250	12.2	13.2	10.0	36.7	3.00
Si 3170	9.0	23.5	14.5	61.3	6.81
Si 1/s	9.5	23.0	14.1	53.2	5.60
Is 304	9.0	24.0	13.2	50.5	5.61
Si 138	9.5	25.0	13.0	60.0	6.31
Si 3315/11	9.0	24.3	12.5	64.2	7.13
Si 2678	10.0	25.2	13.0	60.5	6.05
ES 12	9.5	28.8	13.0	70.2	7.38
Si 0022	9.0	24.4	13.5	60.0	6.66
Si 1050	9.0	28.0	12.9	72.0	8.00
Si 2785	10.5	25.0	13.0	60.5	5.76
Si 0020	9.0	24.5	13.5	60.5	6.72
Si 1523	9.0	23.0	14.1	60.0	6.66
Si 1528	10.5	26.0	13.7	54.8	5.21
Si 1582	10.0	24.5	13.0	60.2	6.02
Si 1780	10.0	24.2	14.5	54.9	5.49
Si 0126	9.0	26.0	14.0	60.2	6.68
RJS 51	9.5	23.2	13.2	60.8	6.40
IS 304	9.2	24.5	13.0	54.4	5.91
Pudukottai	69.1	24.0	13.5	52.5	5.76
TMV 3	9.0	25.5	13.7	66.2	7.13
TMV 4	9.2	24.5	13.5	63.5	6.90
Co 1	10.0	25.0	13.0	54.5	5.45
S. alatum	13.5	9.2	8.5	21.0	1.55

The varieties viz., N 66–250 and N 66–276 (Jakhmola and Yadav, 1974) and TC 289 (Cheema *et al.*, 1982) were already reported to have least infestation.

Most of the cultivable varieties like TMV-3, TMV-4 and CO 1 in Tamil Nadu were found to be highly susceptible under both the conditions tested. Sesame genotypes for resistance to *A. catalaunalis* were evaluated by Aiyadurai *et al.* (1962), Bhattacharjee and Rattan (1962); Krishi *et al.* (1969); Jakhmola and Yadav (1974); Sood *et al.* (1982); Dora and Kamala (1988); Tiwari and Shaw (1988).

Resistant gene to shoot webber from wild species can be transferred to cultivable susceptible varieties using either inter specific hybridization or genetic engineering to evolve resistant lines for effective management.

Sesame lines exhibiting different level of resistance and wild species *S. alatum* were tested along with susceptible cultivars for ovipositional preference in choice and no-choice conditions. There was no oviposition on the wild species under choice situation, while in no-choice condition 0.5 egg/plant was observed. Oviposition in other tested lines varied from 0.7 (Si 1115) to 15.4 (TMV 3) and 3.1 (Si 1115) to 23.2 eggs/plant (Si 3235) in choice and no-choice conditions, respectively (Table 3).

Out of 30 lines tested for larval development of shoot webber, *S. alatum*, ES 22 and Si 250 were found unfavorable exhibiting prolonged larval period, reduction in size, weight, per cent pupation and growth index indicating the antibiosis mechanism (Table 4).

Jotwani *et al.* (1978) concluded that growth index was the most reliable estimate for determining the antibiosis, who also found that larvae of *Chilo partellus* Swinhoe reared on the susceptible sorghum genotypes attained higher weight and length with reduced larval period, higher pupation leading to maximum growth index.

Plants examined for trichome density on leaf showed a direct relationship with ovipositional preference. Wild species *S. alatum* is glabrous without any trichome and the line Si 1115 which was less preferred for oviposition had only 2.1 trichomes per microscopic field (450×). Maximum of 13.5 trichomes/microscopic field was observed in the most preferred TMV 3 line for oviposition. High susceptibility to *A. catalaunalis* by hairy varieties was reported earlier (Anonymous, 1966). The pubescent leaf surface might have provided a better foothold for the female as reported for *H. zea* (Callahan, 1957).

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(Received in September 2001; revised and accepted in May 2002)

ENTOMON **27(4)**: 375–383 (2002)
Article No. ent.27405

Evaluation of the insecticide susceptibility studies of mosquitoes of river Cauvery basin, Karnataka State

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ABSTRACT: This paper summarizes the evaluation of susceptibility studies of different species of mosquitoes to various insecticides at 27 sites of river Cauvery basin including its tributaries (from Thalakaveri to Makedatu, 320 km) for three different seasons in one calendar year. Insecticides supplied by WHO, such as Organochlorines (DDT and Dieldrin), Organophosphates (Parathion, Fenthion and Malathion), Synthetic Pyrethroids (Permethrin) and Carbamate (Propoxur) were used. Complete resistance to organochlorine compounds was observed against all mosquitoes but some of them were fully susceptible for organophosphates, pyrethroids and Carbamates. It was for the first time that such an extensive and systematic study was carried out in the river Cauvery basin.

KEYWORDS: anophelines, culicine, adulticides, larvicides, Cauvery, Karnataka State

INTRODUCTION

The Cauvery basin lies in the State of Karnataka to an extent of 42.2% of its total area of 81 155 sq. km spread over three states (Karnataka, Tamil Nadu and Pondicherry) with its origin in the Western ghats in Coorg district and flows for a length of 320 km out of its total length of 804 km. Its major tributaries in the State are the Hemavathy, Lakshmantheertha, Harangi, Kabini, Swarnawanthy, Lokapavani, Shimsha and Arkavathi. The area of basin in Karnataka is 34 273 sq. km. and covers 18% of the state area comprising of seven Districts *viz.*, Coorg, Hassan, Mysore, Mandya, Chikmagalore, Tumkur and Bangalore rural.

The main objective of this project was to conduct an epidemiological survey of water-borne and water related diseases in the State of Karnataka along the Cauvery basin and its tributaries. Since mosquitoes play an important role in transmitting dreadful diseases such as malaria, filaria, dengue haemorrhages, it became a global priority to control them. Insecticide research led to the 'complete' victories in

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combating pest almost 50 years ago with chlorinated hydrocarbons followed by the organophosphates, methyl carbamates and pyrethroids. Mosquitoes have been shown to develop resistance to virtually all insecticide classes (Metcalf, 1994). Introduction of new insecticides for mosquito control will most likely face the same fate unless integrated resistance management plans are implemented. The development of resistance in the Anopheline mosquito vectors of malaria has disrupted the WHO global malaria eradication programme. The WHO study group of vector control of malaria and other mosquito borne diseases felt that future success of vector control, as a part of global vector control strategy depends upon the systematic review of the available vector options and their selective use (WHO, 1986).

This paper reports on the evaluation of insecticide susceptibility of different species of mosquitoes (*Anopheles and Culex*) at different sites of Cauvery basin and its tributaries for three different seasons of one calendar year. The susceptibility and resistance status of mosquitoes including larvae and adults were studied by following the standardized methods laid by WHO (1981).

MATERIALS AND METHODS

In order to carry out this study, twenty seven sites along the basin were chosen, covering rural, semi-urban and urban areas taking into consideration the socio-economic status of the population. The investigation included the collection of water samples from different sites and analyses for different environmental parameters, like temperature, pH, biological oxygen demand (BOD), nitrite, etc.; identification and summarization of Zooplanktons, Phytoplanktons and Fishes; and biodiversity of mosquito fauna, including vector incrimination studies and insecticide susceptibility studies.

The strains of *Anopheles and Culex* mosquitoes were collected from ponds, pools, tanks and swamps from different surveyed areas along the Cauvery basin. The larvae and pupae were collected from the edge of the pool making a few trial dips with the dipper giving special attention to the edges and floating vegetation. The dipper was gently pressed down and allowed the water to run into it from one side. The larvae found on the surface in the vicinity were collected and transferred to a bowl, then these larvae or pupae were transferred to the bottle by drawing them with a small quantity of water into a wide mouthed pipette. The blood fed adults were collected from human dwellings and cowsheds with the help of an aspirator or by mosquito traps. The adult mosquitoes were identified using Christophers's key (1933) and the larvae were identified following Puri's key (1950).

Full fed adult mosquitoes were used for the adulticide tests immediately after collection from the field. Similarly, the collected late third or early fourth instar larvae were used for larvicide tests. The susceptibility/resistant tests have been carried out where sufficient adults and larvae were available. The different insecticides used for the tests were supplied by the WHO. The insecticides used as larvicides were malathion, fenthion and parathion and the adulticides were DDT, dieldrin, malathion,

propoxur and permethrin. The resistance/susceptibility tests of the mosquitoes were carried out using diagnostic dosage as recommended by WHO (1981).

For larval susceptibility tests, four desired test concentrations (ppm) were prepared for each given insecticide using ethanol. 100 late third instar larvae were exposed to 1 litre of water having 4 ml of test concentration of the given insecticide. One litre of water containing 4 ml of ethanol was used as a control. Mortality was recorded after 24 h of exposure.

The susceptibility tests for adults were carried out using 25 females in the holding tube and after one hour they were transferred to the exposure tube lined with insecticide impregnated paper. Similar procedures were followed for the control. After one hour of exposure, the mosquitoes were released back to the holding tube and the mortality was recorded after 24 h. Per cent mortality was calculated for each set. Abbott's Formula (1925) was applied to correct mortality wherever it was required.

RESULTS AND DISCUSSION

Adulticides

Table 1 shows the list of Anopheline mosquitoes used for adulticides. Among the five species of anophelines, *An. subpictus* showed resistance to DDT and dieldrin (Organochlorines), whereas *An. jeyporiensis* was the only species that showed susceptibility to all above insecticides. *An. subpictus* from Chunchanakatte and Muthathi were susceptible to DDT (60%) and above species from Bannur and Kanakapura (Sangam) showed 90 and 96% susceptibility respectively. All the above species of *Anopheles* were susceptible to malathion, except *An. stephensi* from Kanakapura and *An. hyrcanus* from Holenarasipura which showed resistance by 56.7 and 92% respectively. *An. hyrcanus* from Keralapura, T. Narasipura and Muthathi showed resistance of 80, 95 and 92% respectively to propoxur, while all other anopheline mosquitoes *viz., An. subpictus*, *An. culicifacies*, *An. stephensi*, *An. jeyporiensis*, *An. aconitus* including *An. hyrcanus* from all localities showed full susceptibility. All the anophelines were highly susceptible to permethrin, whereas only *An. hyrcanus* from Keralapura showed 72% resistance.

Three species of *Culex* (*Cx. quinquefasciatus*, *Cx. vishnui* and *Cx.gelidus*) collected from different places (Kushalnagara, Hebbale, Holenarasipura, Muthathi, Bagamandala, Keralapura, T. Narasipura and Thalakadu) (Table 2), have shared full resistance to DDT and dieldrin and have shown full susceptibility to malathion, except the *Cx. quinquefasciatus* from Kushalnagara and Holenarasipura which showed resistance of 72 and 94% respectively. the above mosquitoes are fully susceptible against propoxur and permethrin, except *Cx. gelidus* of Holenarasipura and Muthathi, which showed 81% resistsnce to propoxur and 74% resistance to permethrin respectively.

Larvicides

Four species of *Anopheles viz.*, *An. subpictus*, *An. culicifacies*, *An. stephensi* and *An. hyrcanus* (Table 3) collected from different places were exposed to different

TABLE 1. Susceptibility/resistance among the Anopheline adults of river Cauvery basin—Karanataka

Sl. No.	Places of collection	DDT 4%	Dieldrin 0.4%	Malathion 5%	Permethrin 0.25%	Propoxur 0.1%
	An. subpictus					
1.	Ramanathapura			96% SS		
2.	Keralapura			100% SS		
3.	Chunchanakatte	60% SS		96% SS		
4.	Ramanahally			100% SS		
5.	Sagarakatte			100% SS		
6.	Somanahally			100% SS		
7.	Bannur		90% SS	100% SS		
8.	Thalakadu	84% RR	64% RR		100% SS	96% SS
9.	Chikkamuthathi			90% SS		
10.	Muthathi	60% SS		75% SS		
11.	Kanakapura (Sangama)	84% RR	96% SS	100% SS	96% SS	92% SS
12.	Srirangapatna (Sangama)			100% SS		
	An. culicifacies					
1.	Keralapura	100% RR	100% RR	100% SS	100% SS	100% SS
2.	Holenarasipura	100% RR	100% RR	100% SS	100% SS	100% SS
3.	Chunchanakatte	91.7% RR		89.3% SS		89% SS
4.	T. Narasipura			100% SS	100% SS	
5.	Bannur	90% RR				
6.	Thalakadu			100% SS	100% SS	100% SS
7.	Kanakapura (Sangama)	100% RR	100% RR	100% SS		
	An. stephensi					
1.	Nanjangud	80% RR	70% RR	100% SS	86% SS	80% SS
2.	Kanakapura	65.6% RR	74% RR	56.7% RR	86% SS	100% SS
	An. hyrcanus					
1.	Bagamandala	76% RR	80% RR	60% SS	80% SS	60% SS
2.	Keralapura	96% RR	88% RR	52% SS	72% RR	80% RR
3.	Holenarasipura	100% RR	100% RR	92% RR	100% SS	96% SS
4.	T. Narasipura	100% RR	100% RR	64% SS	100% SS	95% RR
5.	Muthathi	100% RR	100% RR	100% SS	86% SS	92% RR
6.	Kanakapura	100% RR	100% RR	96% SS	100% SS	80% SS
	An. jeyporiensis					
1.	Bagamandala	92% SS	80% SS	100% SS	100% SS	100% SS
2.	Napoklu	88% SS	76% SS	100% SS	100% SS	100% SS
	An. aconitus					
1.	Holenarasipura	100% RR	100% RR	100% SS	100% SS	100% SS

SS: susceptible, RR: resistance.

TABLE 2. Susceptibility/resistance among the Culicine adults of river Cauvery basin—Karanataka

Sl. No.	Places of collection	DDT 4%	Dieldrin 0.4%	Malathion 5%	Permethrin 0.25%	Propoxur 0.1%
	Cx. quinquefasciatus					
1.	Kushalnagara	99% RR	86% RR	72% RR	100% SS	92% SS
2.	Hebbale	100% RR	98% RR	90% SS	100% SS	70% SS
3.	Holenarasipura	68% RR		94% RR		81% RR
4.	Muthathi	91% RR	93.3% RR	84% SS	74% RR	74% SS
	Cx. vishnui					
1.	Bagmandala	68% RR	72% RR	100% SS	96% SS	100% SS
2.	Keralapura	60% RR	53% RR	100% SS	100% SS	93% SS
3.	T. Narasipura	100% RR	100% RR	100% SS	80% SS	60% SS
4.	Thalakadu			100% SS	100% SS	100% SS
5.	Muthathi	100% RR	100% RR	100% SS	72% SS	96% SS
	Cx. gelidus					
1.	Bagamandala	100% RR	100% RR	100% SS	96% SS	80% SS

SS: susceptible, RR: resistance.

concentrations of parathion, fenthion and malathion. It has been shown that *An. subpictus* is fully susceptible to parathion and fenthion in their discrimination dose (0.125 ppm) and sublethal dose (0.001 ppm). However, the same species showed resistance to only parathion in the sublethal doses (0.001 and 0.005 ppm) and fully susceptible to malathion. *An. culicifacies* from Ramanathapura and T. Narasipura showed resistance to parathion in the sublethal dose (0.001 and 0.005 ppm). *An. stephensi* from Nanjangud and Kanakapura and *An. hyrcanus* from Napoklu showed resistance to parathion in the sublethal dose (0.001 ppm). *An. subpictus* from Srirangapatna (Paschimavahini) showed resistance to parathion in the sublethal doses (0.001 and 0.005 ppm). *An. stephensi* from Nanjangud and *An. hyrcanus* from Napoklu showed resistance to malathion in the sublethal dose of 0.025 ppm.

Culex quinquefasciatus collected from different places of Cauvery basin (Table 4) showed different levels of susceptibility or resistance to different insecticides. *Cx. quinquefasciatus* from Kushalnagara, Hebbale, Muthathi, Holenarasipura showed full susceptibility to malathion except Holenarasipura strain which showed resistance (76%) to same insecticide. The same mosquito from Mysore and Kanakapura were susceptible.

Insecticide resistance/susceptibility status in mosquitoes to various insecticides have earlier been reported (Rajagopal, 1977; Curtis and Pasteur, 1981; Vittal *et al.*, 1982; Verma and Rehman, 1984; Brown, 1985; Encinas Grandes and Sagrado, 1988; Manoucehri and Yaghaoobi-Ershadi, 1988; Shridrawi, 1991; Faye *et al.*, 1991; Kulkarni and Naik, 1991; Chakravorthy and Kalyanasundaram, 1992; WHO, 1981, 1986, 1992).

TABLE 3. Susceptibility/resistance among Anopheline larvae of river Cauvery basin–Karanataka

Sl. No.	Places of collection	Parathion (ppm)				Fenthion (ppm)				Malathion (ppm)			
		0.001	0.005	0.025	0.125	0.001	0.005	0.025	0.125	0.025	0.125	0.625	3.125
	An. subpictus												
1.	Napoklu	100% SS	100% SS	100% SS	100% SS	100% SS	100% SS	100% SS	100% SS				
2.	Kushalnagara	100% SS	100% SS	100% SS	100% SS	100% SS	100% SS	100% SS	100% SS				
3.	Ramanathpura	66.7% SS	100% SS	100% SS	100% SS	90% RR	100% SS	100% SS	100% SS				
4.	Keralapura	100% SS	100% SS	100% SS	100% SS	100% SS	100% SS	100% SS	100% SS				
5.	Holenarasipuara	100% SS	100% SS	100% SS	100% SS	100% SS	100% SS	100% SS	100% SS				
6.	Chunchunakatte	100% SS	100% SS	100% SS	100% SS	100% SS	100% SS	100% SS	100% SS				
7.	Ramanahally	90% SS	100% SS	100% SS	100% SS	100% SS	100% SS	100% SS	100% SS				
8.	Srirangapatna (Sangama)	100% SS	100% SS	100% SS	100% SS	100% SS	100% SS	100% SS	100% SS				
9.	Srirangapatna (Paschimavahini)	92% RR	88% RR	100% SS	100% SS	100% SS	100% SS	100% SS	100% SS	84% SS	88% SS	100% SS	100% SS
	An. culicifacies												
1.	Ramanathpura	100% RR	94% RR	100% SS	100% SS	100% SS	100% SS	100% SS	100% SS	62% RR	100% SS	100% SS	100% SS
2.	Chunchanakatte	100% SS	100% SS	100% SS	100% SS	100% SS	100% SS	100% SS	100% SS	100% SS	100% SS	100% SS	100% SS
3.	T. Narasipura	96% RR	84% RR	100% SS	100% SS	100% SS	100% SS	100% SS	100% SS	92% SS	84% SS	100% SS	100% SS
4.	Shivanasamudra	50% RR	95% SS	100% SS	100% SS	100% SS	100% SS	100% SS	100% SS	100% SS	100% SS	100% SS	100% SS
	An. stephensi												
1.	Nanjangud	80% RR	100% RR	100% SS	100% SS	100% SS	100% SS	100% SS	100% SS	100% RR	92% SS	100% SS	100% SS
2.	Kanakapura	100% RR	100% SS	100% SS	100% SS	100% SS	100% SS	100% SS	100% SS	100% SS	100% SS	100% SS	100% SS
	An. hyrcanus												
1.	Napoklu	80% RR	100% SS	100% SS	100% SS	100% SS	100% SS	100% SS	100% SS	100% RR	100% SS	100% SS	100% SS

SS: susceptible, RR: resistance.

TABLE 4. Susceptibility/resistance among the Culicine larvae of river Cauvery basin—Karnataka

Sl. No.	Places of Collection	Parathion (ppm)				Fenthion (ppm)				Malathion (ppm)			
		0.001	0.005	0.025	0.125	0.001	0.005	0.025	0.125	0.025	0.125	0.625	3.125
	Cx. quinquefasciatus												
1.	Kushalnagra									80% SS	100% SS	100% SS	100% SS
2.	Hebbale									72% SS	100% SS	100% SS	100% SS
3.	Holenarasipura									76% RR	100% SS	100% SS	100% SS
4.	Mysore	100% RR	100% SS	100% SS	95.2% SS	83.2% RR	100% SS	100% SS	100% SS				
5.	Muthathi									84% SS	86% SS	92% SS	100% SS
6.	Kanakapura	88% RR	100% SS	100% SS	100% SS	86% RR	100% SS	100% SS	100% SS	100% SS	100% SS	100% SS	100% SS

SS: susceptible, RR: resistance.

Our laboratory is currently performing genetic studies towards the eventual development of strains for genetic control of the vectors of infectious diseases. An attempt has been made to evaluate the genetic basis of insecticide resistance of the mosquitoes of South Indian region (Rao and Shetty, 1992; Rajasree and Shetty, 1998; Ghosh and Shetty, 1999; Priyalakshmi *et al.*, 1999). The insecticide resistance gene has been used for the preferential elimiation of females in the genetic sexing system of *Cx. quinquefasciatus* (Shetty, 1987).

In our present investigation, the data clearly showed that most of the mosquitoes including *Culex* and *Anopheles* showed resistance to DDT and dieldrin. Some of the Culicine larvae were fully susceptible to parathion (0.125 ppm), fenthion (0.125 ppm) and malathion (3.125 ppm). However, larvae of *Cx. quinquefasciatus* collected from Mysore and Kanakapura showed resistance to parathion and fenthion, when exposed to the sublethal dose (0.001 ppm). Most of the anopheline mosquitoes showed susceptibility to parathion (0.125 ppm), fenthion (0.125 ppm) and malathion (3.125 ppm). However, some strains from Srirangapatna *(An. subpictus)*; Ramanathapura and T. Narasipura *(An. culicifacies)*; Nanjangud and Kanakapura *(An. stephensi)* and Napoklu *(An. hyrcanus)* showed resistance to parathion when exposed to sublethal concentration (0.005 ppm). Most of the anopheline adults were resistant to DDT (4%) and dieldrin (0.4%), whereas they were fully susceptible to malathion (5%), propoxur (0.1%) and permethrin (0.1%).

From the above survey, it revealed that most of the important mosquito species (both *Anopheles* and *Culex*) available in surrounding areas of Cauvery basin were fully resistant to Organochlorines (DDT and dieldrin), very less resistant to Organophosphate insecticides (parathion, fenthion and malathion) and fully susceptible to Carbamate (propoxur) and Synthetic pyrethroid (permethrin). Thus, synthetic pyrethroid could be used for the control of mosquitoes in the above said surveyed regions. This would serve as a guideline to the Public Health Department for controlling mosquito vectors using appropriate recommended insecticides.

ACKNOWLEDGEMENTS

The author is thankful to the financial assistance by the Ministry of Environment and Forests, Government of India, New Delhi and the Co-ordination of Madras Science Foundation, Chennai, for carrying out this Epidemiological studies under the 'Integrated Environmental Programme of Research on Cauvery basin, Karnataka'. The author also acknowledges the technical support of UGC (DSA Programme), New Delhi.

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(Received in September 2001; revised and accepted in May 2002)

ENTOMON 27(4): 385–392 (2002)
Article No. ent.27406

Preliminary observations on the buffering mechanism in the midgut of larva of *Oryctes rhinoceros* (Coleoptera: Scarabaeidae)

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ABSTRACT: The alimentary canal of larvae of *Oryctes rhinoceros* is characterized by high alkalinity as revealed by feeding cellulose-pH indicator diet. The pH in the anterior midgut is between 10 and 10.2 and that of the posterior half of the midgut being 8.4–9.8. An attempt is made to study the possible regulation of midgut lumen pH by the midgut epithelial factors. Empty ligated posterior midgut tubes, injected with thymol blue-buffer solution of pH 10.2 or pH 7 were incubated with 2 ml of midgut epithelial extract in a bioassay apparatus, bubbling oxygen and the colour of the gut contents was monitored at 1 min interval. The controls were incubated with insect saline. In midgut preparations injected with thymol blue buffer solution of pH 10.2, the colour of the gut contents changed from blue to yellow indicating a decrease in pH to <7.4 after 17.56 ± 6.00 min (Control 31.25 ± 7.82 min). In midgut preparations injected with thymol blue buffer solution of pH 7, no colour change was observed at 60 min as in controls. The analysis of midgut contents revealed the presence of cations such as Na^+, K^+, Ca^{2+}, Mg^{2+} and anions like HCO_3^-, CI^-, and PO_4^{3-}. The posterior half of the midgut has a higher concentration of ions involved in lowering pH. It is assumed that the secretion these ions may be stimulated by a factor present in the midgut epithelium.

KEYWORDS: *Oryctes rhinoceros*, midgut, gut pH

INTRODUCTION

In insects, the pH of the alimentary canal has significance in the spatial organization of digestion. Previous studies have provided valuable measurements of pH ranges in the different segments of the alimentary canal in various insects (Craig and Clark, 1938; Hastings and Pepper, 1943; Leven book, 1950a,b) but with very little insight into the actual mechanisms of pH regulation. We have undertaken a detailed study on the buffering mechanism and possible regulation of gut pH by insect midgut endocrine

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system in the larvae of the coconut pest *Oryctes rhinoceros*. The preliminary findings of the study are reported in this paper.

MATERIALS AND METHODS

Third instar larvae of *Oryctes rhinoceros* were reared following the method of Sreekumar (1998) using rotting coconut logs.

pH of gut contents in the different regions of alimentary canal of third instar larvae of *O. rhinoceros* were determined by feeding a 1000 : 1 mixture of cellulose-pH indicator dyes (alizarin yellow, alizarin red, phenol red, thymol blue, bromothymol blue, metacresol purple, orthocresolpthalein, thymolpthalein and brilliant yellow) mixed with 10 ml distilled water for 48 hrs (Dadd, 1975). The larvae were dissected and the pH of the contents in different regions of the gut were recorded by comparing the colour in different segments of the alimentary canal with the colour of the indicator dyes at known pH.

For analysis of inorganic ions, the posterior midgut region of the larva fed on cellulose for 72 hr was dissected out, washed thoroughly with double distilled water and blotted dry. The gut wall was slit open longitudinally and the contents were transferred to 5 ml double distilled water. The contents were homogenized in a glass homogenizer and centrifuged at 10000 rpm for 10 min at 4 °C The supernatent was collected and made up to 5 ml.

Quantitative estimation of inorganic ions were done following the method of Franson (1995). Cation analysis was performed in a flame photometer. Chloride estimation was done using Orion Expandable ion analyzer ED 940. The concentration of Mg^{2+} and HCO^{3-} was estimated by titrimetry. For Mg^{2+} analysis, 1 ml sample having 50 times dilution mixed with 1 ml NH_3–NH_4Cl buffer and 10 mg of eriochrome T indicator was titrated against 0.02 M EDTA. End point is the appearance of steel-blue colour. The concentration of HCO_3^- was estimated using 1 ml sample having 50 times dilution mixed with 2 drops of methyl orange indicator. It was titrated against 0.02 M H_2SO_4. End point is the appearance of golden yellow colour. Phosphorus concentration was determined by spectrophotometric method, measuring OD at 880 nm.

0.1% solutions of thymol blue was prepared in glycine-NaCl–NaOH buffer having pH 10.2 and citrate–phosphate buffer having pH 7. For preparation of midgut epithelial extract thoroughly cleaned epithelial tissues from final instar larvae were boiled in insect saline [7 g sodium chloride, 0.2 g calcium chloride, 0.2 g potassium chloride, 0.2 g sodium bicarbonate and 0.1 g dextrose in 1000 ml distilled water] for 10 min, centrifuged at 10000 g for 10 min at 4°C. The supernatant obtained was made up with insect saline to a concentration equivalent to 2 midgut epithelia/10 ml insect saline.

The posterior midgut was selected for studying the effect of epithelial extracts on regulation of pH in the bioassay because the anterior midgut had a dark brown secretion which masked the pH indicator dye. The alimentary canal was dissected out in insect saline and the posterior half of the midgut was separated and washed thoroughly in 4–5 changes of insect saline. One end of the posterior midgut was

TABLE 1. Concentration of ions in the gut contents of larvae of *Oryctes rhinoceros*

Ions	Concentration of ions in midgut (μg/ml)	
	Anterior half	Posterior half
Na^+	40.625 ± 18.61 (8)	52.625 ± 22.23 (8)
K^+	324.875 ± 101.327 (8)	721.125 ± 164.028 (8)
Ca^{2+}	41.25 ± 16.739 (8)	313.125 ± 99.798 (8)
Mg^{2+}	34.338 ± 15.096 (8)	29.13 ± 6.126 (8)
Cl^-	493.5 ± 146.189 (8)	624.5 ± 141.945 (8)
HCO_3^-	945.5 ± 203.623 (8)	1128.5 ± 389.910 (8)
PO_4^{3-}	159.625 ± 50.578 (8)	183.813 ± 63.700 (8)

Values are presented as mean ± SD for number of observations indicated between parenthesis.

TABLE 2. Effect of midgut epithelial extracts on the maintenance of lumen pH in ligated preparations of posterior midgut of larvae of *Oryctes rhinoceros*. The concentration of the extract was two midgut epithelia/10 ml insect saline

pH indicator buffer solution	Change in color	Time required for change in colour (min) with varying concentration	
		Test	Control
Thymol blue glycine NaCl–NaOH buffer, pH 10.2	Blue (pH 10.2) to Yellow (pH < 7.4)	17.56 ± 6.00* (8)	31.25 ± 7.82 (8)
Citrate–phosphate buffer, pH 7	Yellow (pH 7) to Purple (pH > 8.4)	No change	No change

Each value is mean ± SD of eight observations. *Significant at 0.01 level.

ligated using hair. Through the other end of the tube, 0.06 ml of pH indicator-buffer solution (pH 10.2 or 7) was injected. As the needle was withdrawn the loop of hair placed already at this end was tautened and tied. The midgut tubes thus filled with pH indicator-buffer solutions were used for bioassay.

The midguts thus prepared were incubated with 2 ml midgut epithelial extract in a bioassay apparatus (Sunitha *et al.*, 1999). Controls were incubated in insect saline. Time taken for change in colour of the gut contents from blue to yellow was noted at 1 min interval (Table 2).

RESULTS AND DISCUSSION

The alimentary canal of larvae of *Oryctes rhinoceros* shows morphological similarity with that of larvae of the closely related species, *Oryctes nasicornis* (Bayon, 1980) in having a cylindrical midgut, with caeca arranged in the form of rings around the anterior, posterior and some what middle regions of the midgut (Fig. 1). The hind gut is characterised by the presence of a fermentation chamber called proctodaeal dilation.

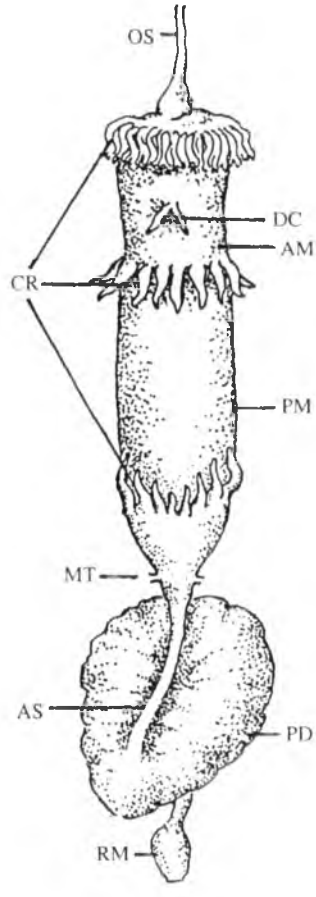

FIGURE 1. Alimentary canal of larvae of *Oryctes rhinoceros* AM – Anterior midgut; AS – Anterior sphincter; CR – Caecal ring; DC – Dorsal caeca; MT – Malpighian tubule; OS – Oesophagus; PD – Proctodaeal dilation; PM – Posterior midgut; RM – Rectum.

The alimentary canal of *Oryctes rhinoceros* shows high alkalinity as in *Oryctes nasicornis* (Bayon, 1980) Alkaline gut pH is reported in many lepidopteran larvae which may be an adaptive mechanism that facilitates the digestion of proteins which are complexed with tannins, lignins and other polyphenols commonly present in many types of foliage (Berenbaum, 1980). Detritus feeding dipteran and scarabaeid larvae feed on food materials having low nutritive quality. While proteins contained in the detritus food remain complexed with tannins and lignins, most of the carbon is present in polymers such as cellulose, hemicellulose and lignin (Martin *et al.*, 1980; Bayon, 1980). In these insects protease and carbohydrase activities require highly alkaline pH. As well as affecting the activity of the insect's own enzymes, the pH of the midgut influences the potentially harmful effects of some ingested compounds (Felton *et al.*,

1992). In most insects, pH often varies along the length of the gut. In the foregut it is greatly influenced by the food and varies with diet. Although midgut pH may also vary with the diet, it is usually buffered to maintain a relatively stable level. However, variation may occur along the length of the midgut. The present study reveals that the alimentary canal of the larvae of *Oryctes rhinoceros* is characterised by high alkalinity. A gradual decrease in pH was observed in the posterior half of the midgut, proctodaeal dilation and rectum. The whole gut stained blue with bromothymol blue and white with thymolpthalein suggesting the lower and upper limits of pH in the gut to be 7.4 and 10.2 respectively. The foregut and anterior half of the midgut showed high alkalinity with pH between 9.2 and 10.2. This region stained magenta with alizarin red and purple with metacresol purple. Between the anterior region and the region of the posterior row of midgut caeca pH was between 8.3 and 8.8. In the posterior midgut behind the region of the caecal rings pH declined to 7.9. The proctodaeal dilation showed pH from 7.9 to 8.4. pH was between 7.4 and 7.5 in the rectum.

It is reported in *Oryctes nasicornis* that the posterior midgut and the proctodaeal dialation are colonized by a variety of symbionts which are responsible for cellulose digestion (Bayon, 1980). The anaerobic digestion of many polymeric organic fibres such as cellulose is completed in three stages. In the first stage cellulose decomposing organisms convert cellulose into soluble compounds by the action of facultative microorganisms. In the second stage acid bacteria and other microbes convert it into organic acids. Organic acids are converted to methane and carbon dioxide by means of methane bacteria, which are strictly anaerobic, during the final stage. The rate of cellulolysis is influenced by factors like substrate, bacterial concentration, pH and temperature. In *Oryctes nasicornis*, the mesenteron is found to be the site of first stage of cellulolysis (Bayon, 1980). One of pre-cellulolytic condition is basic media (Hungate, 1966). In *Oryctes rhinoceros* pH is strongly basic in the anterior half of the midgut as in *Oryctes nasicornis* (Bayon, 1980) and this may favour pre-cellulolysis. The posterior half of the midgut of the larvae of *Oryctes nasicornis* is reported to be the site of production of volatile fatty acids especially acetic acid. As a consequence a gradual fall in pH occurs along the midgut (Bayon, 1980; Bignell *et al.*, 1989; Martin *et al.*, 1980). In the present study a similar decline in pH is observed along the alimentary canal in larvae of *Oryctes rhinoceros*, probably indicating acid production. Methogenic bacteria found in the proctodaeal dilation of scarabaeid larvae may use acetic acid for final conversion into CO_2 and methane. In *Oryctes rhinoceros* larvae the proctodaeal chamber or fermentation chamber shows a further lowering of pH to 7.9 in agreement with the observation in *Oryctes nasicornis* (Bayon, 1981). This lowering of pH may be partly due to the release of uric acid by Malpighian tubules.

In the present study the midgut contents of larvae of *Oryctes rhinoceros* have been analysed for quantitative estimation of important buffering ions. The results revealed the presence of cations such as Na^+, K^+ , Ca^{2+} and Mg^{2+} and anions like HCO_3^-, Cl^- and PO_4^- in the gut contents. The posterior half of the midgut has a higher concentration of these ions when compared with the anterior half (Table 1). Secretion of electrolytes and water provides the fluid and pH necessary for digestion. Under

normal condition, biochemical reactions tend to maintain pH in the proper range automatically. Ion transport in the midgut has only been investigated in a very small number of insects. The alimentary canal must continuously assimilate inorganic ions and water of dietary origin, in order to replace their continuous loss. A major amount of the total energy utilized in the absorption of electrolytes and water is directed towards the reabsorption of secretions in the hindgut region. One of the most thorough studies of ion movements in the midgut has been done in the isolated midgut of *Hyalophora cecropia*. The midgut of this phytophagous insect actively transports K^+ from the haemolymph into the gut lumen (Harvey and Nedergaard, 1964). In *Calpodes ethlius* the Malpighian tubules are involved in the secretion of sodium and potassium into the gut lumen (Irvine, 1969). The concentration of a given ion in gut contents is dependent upon its quantity in the diet, the quantity added by way of secretions of the digestive system and quantity absorbed relatively to the volumes of water ingested, secreted or absorbed. In *Oryctes rhinoceros* the concentration of both cations and anions except Mg^{2+} is higher in the posterior midgut. Posterior midgut of scarabaeid larvae being the region of cellulolytic activity by acid bacteria, volatile fatty acids are formed as free acids with a pK of 4.8 (Bayon, 1981). The pH of the midgut contents varies inversly with the rate of volatile fatty acid production. However, the pH can be maintained in the normal range by the secretion of HCO_3^- by gut epithelium and absorption of volatile fatty acid as in ruminants (Hungate, 1968; Bryant, 1977; Phillipson, 1977). In ruminants microbial fermentation would be favoured by the release of HCO_3^- and retention of water in the lumen of hind gut. Absorption of volatile fatty acid is accompanied by a simultaneous increase in the secretion of HCO_3^- and decrease in the pCO_2 level of lumen contents. This probably explains the presence of a higher concentration of bicarbonate in the posterior midgut in larvae of *Oryctes rhinoceros*. Dow (1981) has observed that secretion of HCO_3^- across the apical membrane and its subsequent conversion to CO_3^{2+} may provide the main anion both for balancing K^+ and for titrating the lumen contents to the high pH. Most phytophagous insects have a lower concentration of sodium than insects with other feeding habits. On the other hand, K^+ and Mg^{2+} levels tend to be higher in phytophagous groups reflecting the levels of these elements in plant tissues. In the present study, larvae of *Oryctes rhinoceros* were fed on cellulose for 72 hr before measuring the concentration of various ions. It, thus, appears that ions involved in buffering mechanism in this insect are not obtained directly from food, but are secreted by the epithelium. Goblet cells of coleopterans and lepidopteran larvae are known to be involved in the secretion of K^+ into the gut lumen (Chapman, 1998). PO_4^{3-} production may take place as a by product of microbial digestion (Alexander, 1962).

An earlier study in *Rhynchophorus ferrugineus* larvae has shown that a factor present in the midgut epithelium is capable of maintaining pH possibly by stimulating epithelial cells to release secretions of buffering nature into the lumen of the gut (Sunitha *et al.*, 1999). The effect of midgut epithelial factor is both time and dose dependent which indicate that it could be an endocrine factor. The present study shows that such a factor is present in *Oryctes rhinoceros* as well. The results indicate that the

posterior midgut is concerned with the secretion of buffering ions, for lowering pH and regulating pH changes resulting from the activity of acid bacteria. No regulation was observed in the opposite direction (Table 2). The midgut epithelium of larvae of *Oryctes rhinoceros* contains endocrine cells reminiscent of α- and β-cells of islets of Langerhans of vertebrates (Sreekumar, 1998). It is reported earlier that a midgut factor (hormone) is involved in releasing digestive enzyme from the epithelium into the midgut lumen. The midgut epithelial factor regulating pH in midgut preparations in the event of altered pH conditions, and thus chemical changes of lumen contents, could also be a hormone. Further studies involving characterization, isolation and purification of this factor may be valuable in answering the question whether the midgut factors stimulating digestive enzyme release and regulating gut pH are one and the same or different.

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(Received in October 2001; revised and accepted in May 2002)

ENTOMON 27(4): 393–401 (2002)
Article No. ent.27407

Morphology and distribution of sensilla in palp and leg appendages of larva and nymph of *Argas persicus* Oken (Acarina: Argasidae)

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ABSTRACT: Morphology and distribution of sensilla in palp and leg appendages of larva and nymph of *Argas persicus* Oken were studied. The total number of sensilla on palp of larva and nymph were found to be 20 and 35 respectively. The total number of sensilla on various leg appendages of larva were 38, 30, 31 in Ist, IInd and IIIrd legs respectively, while the total number of sensilla counted in nymphs were 80, 63, 71, 71 in Ist, IInd, IIIrd and IVth leg appendages respectively. Chemosensilla are more in number than mechanosensilla. The sensilla population on palps and leg appendages progressively increases from larva to nymph. No marked difference in the number of denticles on hypostome were observed in the post embryonic developmental stages. However, the denticles were found to be more developed in larva than in nymph and adults.

KEYWORDS: Acarines, hypostome, scanning electron microscopy

INTRODUCTION

Among arthropods, acarines are most successful land dwellers with the result they are widely dispersed, highly motile, heterotrophic organisms with diverse habit and habitats. They owe this achievement largely to their cuticle, which provides mechanical support and protection from the water loss. Their sense organs also need special and unique adaptation to remain and receptible to environmental stimuli.

During the last three decades increasing interest in communication system of acarines and other insect pests have been developed with a view to environmentally safe method for the control of pest populations (Rao *et al.*, 1990; Kumar *et al.*, 1984, 1992, 1995; Axtell, 1999).

Ticks use number of sensory array for host location, feeding and search for mate

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(Waladde, 1987). A fair amount of work on sensilla on the tarsi and forelegs have been studied in detail in various species of ticks (Foelix and Axtell, 1971, 1972; Roshdy *et al.*, 1972; Roshdy and Marzouk, 1984; Clifford *et al.*, 1983; Hoogstraal *et al.*, 1983; Klompen and Oliver, 1993). Studies on sensilla mapping and their projections to the central nervous system in adult *Argas persicus*, a major poultry pest in India have been reported (Sridharan *et al.*, 1998). Since sensory inputs for chemosensory perception varies in different developmental stages of the same species, the present study was undertaken to examine the sensory equipment of palps and legs of larva and nymph of *A. persicus* and the variation in distribution of sensilla on the palps and leg appendages of larva and nymph of *A. persicus*.

In this study the mapping of sensilla has been carried out by light microscopy using ethanolic silver nitrate staining. The surface morphology of hypostome was observed by scanning electron microscope (SEM).

Test insect

A. persicus Oken larvae and nymphs were collected from the Goverment Poultry Farm, Gwalior and maintained at the room-temperature (RT) under photoperiodic condition (L : D 12 : 12 hrs) and fed on chicken blood according to the technique described by Rau (1966).

Mapping of sensilla

The larvae and nymphs were immersed overnight in 15 ml of 0.5% silver nitrate solution prepared in 70% ethanol and kept in diffuse light (Venkatesh and Singh, 1984). The specimen were dehydrated with grades of ethanol cleared in xylene. Each appendage was mounted with DPX under a glass coverslip. Slides were examined under a Leitz Diaplan Microscope and photographed. The porous sensilla stained dark brown (Venkatesh and Singh, 1984). Reconstruction of the maps showing locations of various sensilla was carried out under a Leitz Diaplan Microscope fitted with camera lucida.

Scanning electron microscopy

The palp and leg appendages were fixed for 24 hrs in 2.5% glutaraldehyde solution prepared in 0.1M sodium phosphate buffer of pH 7.4. Following fixation, specimen were dehydrated with ethanol, cleared in xylene and dried at room temperature for 24 hrs (Prakash *et al.*, 1995). They were then mounted on stubs, coated with a thin layer of gold in a JFC 1100 sputter coating unit and examined in a Jeol 840 scanning electron microscope between 5–10 KV.

Sensilla mapping on palps and leg appendages

Silver staining technique was used for mapping distribution of various types of sensilla present on the palp and leg appendages. Argyrophilic sensilla were deeply stained (dark brown) and are believed to be chemosensory. The non-argyrophilic (unstained)

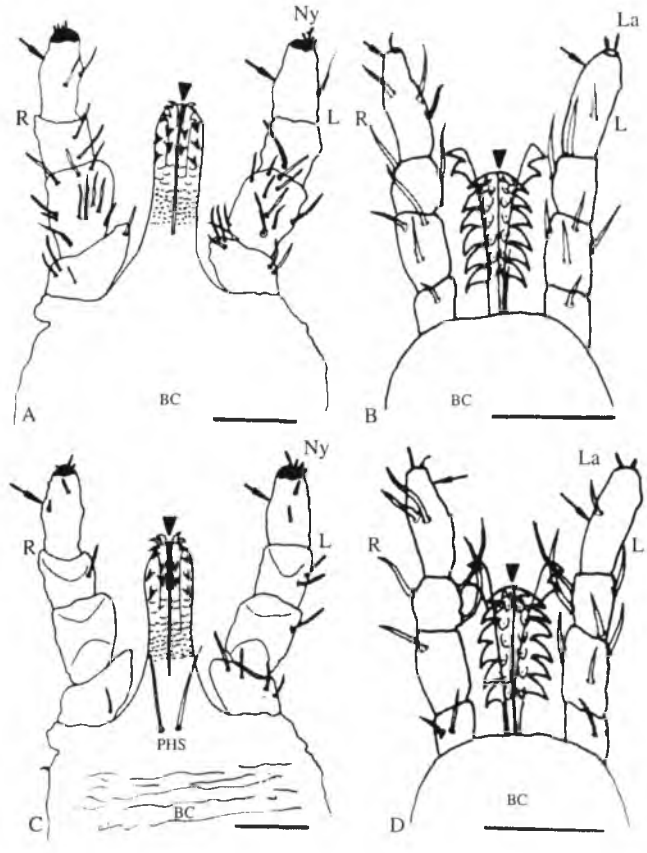

FIGURE 1. Camera lucida diagram of mouth parts of larva and nymph of *Argas persicus* showing the sensilla on palps and basis capitulum. (A) Dorsal view of nymph mouth parts. (B) Dorsal view of larva mouth parts. (C) Ventral view of nymph mouth parts. (D) Ventral view of larva mouth parts. Argyrophilic hairs=solid filled hairs; Hypostome with denticles=arrow head; Larva=La; Left palp=L; Nymph=Ny; Mechanosensory hairs=shown as hollow hairs; Palp=Arrow: Post-hypostomal sensilla=PHS; Right palp=R; Scale Bar=100 um.

sensilla are considered to be mechanosensilla. The schematic diagram of sensillary maps reconstructed from camera lucida of the whole mounts showing the distribution of argyrophilic and non-argyrophilic sensilla on palp and leg appendages of larva and nymph are shown in [Fig. 1(A), (B), 2 and 3]. The sensilla counts for larva and nymph are given in Table 1.

The ventral side of basis capitulum of larva was shown to bear no sensilla [Fig. 1D] while in nymphs a pair of long sensory hair post hypostomal sensilla (PHS) was observed [Fig. 1C]. There are no sensilla on the dorsal side of the basis capitula of larva and nymph [Fig. 1(A) and (B)]. The limited number of sensilla present in

TABLE 1. Number and the distribution of sensilla on basis capituli, palp and legs of the larva and nymph *Argas persicus*

Body parts	Larva		Nymph	
	Chemosensilla	Mechanosensilla	Chemosensilla	Mechanosensilla
Basis capituli	0	0	1	2
palp				
Segments				
1	1	1	2	2
11	1	1	5	3
111	2	2	4	3
1V	10	2	11	2
First Leg				
Coxa	0	0	0	0
Trochanter	2	0	2	0
Femur	5	1	12	3
Tibia	4	1	13	3
Metatarsus	3	1	14	3
Tarsus*	14	2	23	3
Haller's organ*	5	0	4	0
Second leg				
Coxa	0	0	0	0
Trochanter	0	1	2	0
Femur	4	2	10	3
Tibia	4	1	9	3
Metatarsus	4	1	10	3
Tarsi	10	3	20	3
Third Leg				
Coxa	0	0	0	0
Trochanter	1	0	2	0
Femur	1	0	10	4
Tibia	3	2	13	3
Mrtatarsus	2	2	14	1
Tarsi	13	2	22	2
Fourth Leg				
Coxa			0	0
Trochanter			2	1
Femur			9	5
Tibia			9	5
Metatarsus			12	4
Tarsi			21	3

Average total number of sensilla from counts of five specimens for larva = 119 ± 4 & for nymph = 318 ± 16.
Excluding Haller's organ. 11

the basal segment are few while the terminal segment has a group of argyrophilic chemosensilla. Quantitative studies have shown that chemosensilla are more in number than mechanosensilla. The total number of sensilla (chemo and mechano) on

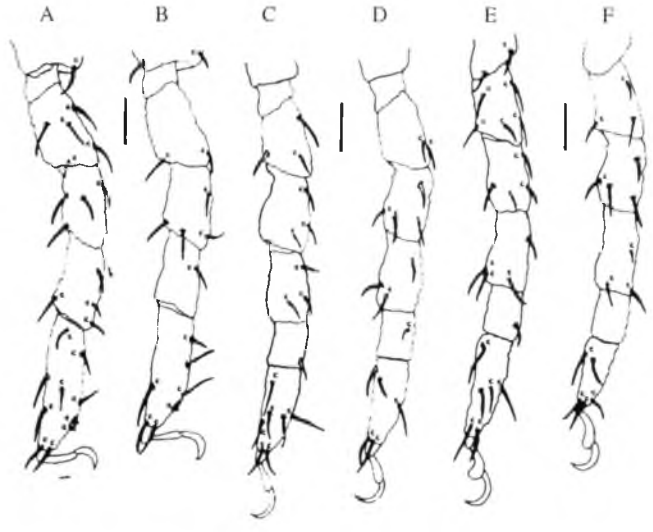

FIGURE 2. Digrams showing distribution of sensilla on the surface of legs of larva *Argas persicus*. The argyrophilic hairs have been marked by letter 'C' and nonargyrophilic hairs have been left unmarked. (A) Dorsal view of first leg; (B) Ventral view of first leg; (C) Dorsal view of second leg; (D) Ventral view of second leg; (E) Dorsal view of third leg; (F) Ventral view of third leg. Scale bar=100 μm.

palp of larva and nymph were 20 and 35 respectively. The number of sensilla on palps increases from larva to nymph.

Figures 2 and 3 presents the reconstructed camera lucida diagrams showing the number and location of sensilla on the legs of *A. persicus*. It shows that coxa rarely has any sensilla while on the other segments, sensilla are distributed in increasing trend towards distal segment. Similarly the number of chemosensilla are more than mechanosensilla. Dorsodistal segment of the tarsus-1 of the first leg has a specialised structure called Haller's organ in which are concentrated mainly argyophilic sensilla. The total number of sensilla on various leg appendages of larva were 38, 30, 31 in Ist, IInd, and IIIrd leg respectively. While the total number of sensilla in nymph were 80, 63, 71, 71 in Ist, IInd, IIIrd, IVth leg appendages respectively (Table 1). The sensilla located on mouth parts and leg appendages provide excellent tools through which ticks perceive environmental stimuli and are used to locate host as well as mate(s).

It has been observed that the variation in sensilla population depends upon the habit and habitat of the organism which inturn determine the response of the species to respective stimuli. Parashar *et al.* (1994) reported that in comparison to biting muscids and mosquitoes the horsefly has a large number of sensilla and perhaps is better conditioned to travel longer distances in search of host. The large number of sensilla in these tabanids might be an adaptation for locating hosts at longer distances as the olfactory activity is directly related to the number of sensilla. *A. persicus*, a crawling haematophagus acarine has comparatively reduced number of sensilla possibly due

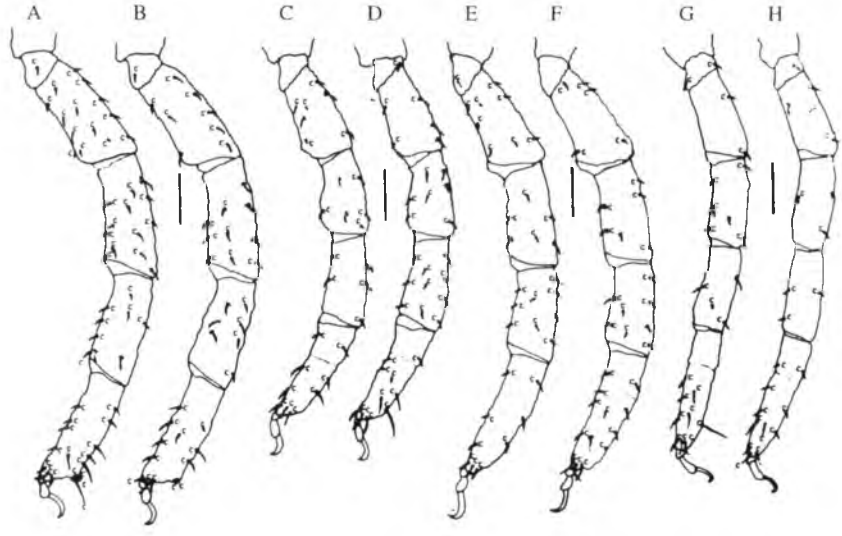

FIGURE 3. Diagram showing the distribution of the sensilla on the surface of the legs of *Argas persicus* nymph stage. Sensory hairs in this diagram have been accentuated in width by approximately 50% to make them appear clearly. In addition, the argyrophilic hairs have been marked by the letter 'C' and the non-argyrophilic hairs have been left unmarked. (A) Dorsal view of first leg; (B) Ventral view of first leg; (C) Dorsal view of the second leg; (D) Ventral view of the second leg; (E) Ventral view of the third leg; (F) Dorsal view of the leg; (G) Ventral view of the fourth leg; (H) Dorsal view of the fourth leg. Scale bar=200 um.

to parasitic nature of life and near by location of their host. Similar observations on the reduced number of sensilla in bed bugs *Cimex lectularis* has been reported by Steinbrecht and Muller (1976) and in *Cimex hemipterus* by Singh *et al.* (1996).

Scanning electron microscopic studies

Scanning electron micrographs given in Fig. 4 on palps of larva, nymph and adult showed some interesting features observed in the hypostome of larva, nymph and adult of *A. persicus*. There was no marked difference in the number of denticles during post-embryonic development. However, denticles were found to be more developed in larva than in nymph and adult. The proximal part of hypostome attached to basis capitulum has reduced hypostomal teeth in the post larval stages. While the distal part of hypostome has fully developed hypostomal teeth (Fig. 4). Cheliceral digits and hypostomal teeth have been reported to serve as cutting and attachment tools required for the process of attachment and blood sucking from the host body (Soneshine *et al.*, 1984). Tick cuts the host's integument and the chelicerae and hypostome are simply thrust into the cut wound. While in the ticks with short mouth parts cutting into the host integument is accompanied by laying a cement cone which binds the tick on to host. In both cases, however, the palps with the sensilla are kept on the host body surface, they never enter with the feeding lesion (Waladde, 1987). Arthur (1962) reported that

FIGURE 4. Scanning electron micrographs showing mouth parts of different stages of *Argas persicus*. (A) larva; (B) Nymph, paired palps and (C) Hypostome of adult, BC=Basis capituli; PHS=Post hypostomal sensillum; PPS=Post palpal sensillum. Scale bar=10 um in panel A and 50 um in panel B and C.

larva feeds for longer time than nymph and adult with average feeding time of 2–3 days, 24 hrs and 45–60 minutes respectively. Possible the tiny larva has well developed hypostomal teeth to keep lodging for longer and stronger attachment for blood sucking than nymph and adult.

In the present study, in essence we have determined the number, distribution, surface morphology of prominent sensilla located on the palp and leg appendages of larva and nymphs of *A. persicus*.

ACKNOWLEDGEMENTS

The authors are thankful to Dr. R. V. Swamy, Chief Controller Resarch and Development at R&D HQrs, New Delhi and Shri K. Sekhar, Director, Dr. D. K. Jaiswal, Associate Director, DRDE, Gwalior for encouragement and interest in the work during the course of study.

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(Received in November 2001; revised and accepted in April 2002)

ENTOMON 27(4): 403–409 (2002)
Article No. ent.27408

Butterflies of Barda wildlife sanctuary, Gujarat

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ABSTRACT: Butterfly survey of the sanctuary was conducted during August, 1999 to July, 2000. A total of 59 species and two additional female forms of butterflies belonging to eight families were recorded during the study period. Out of them six species are abundant, 20 species are common, 20 species are uncommon and 13 species are rare in the sanctuary. Seasonal variation in butterfly species was observed. There ware 44 species in monsoon which increased to 47 in winter but decreased to 17 in summer. However, 14 species were recorded throughout the year.

KEYWORDS: Butterflies, Barda Wildlife Sanctuary

INTRODUCTION

The Barda Wildlife Sanctury was a private forest of the Ex-princely states of Porbandar and Jamnagar. The santuary (Fig. 1) having a latitude 21°40′–21°55′ north and longitude 69°40′–69°50′ east, is spread over 192.31 sq km. North part of the sanctuary is known as Jam Barda and South part of the sanctury is known as Rana Barda.

Very little is known regarding butterflies of the Barda Wildlife Sanctuary (BWS), which provides inventory and status of its. The authors explored the entire sanctuary area at all elevations for a period of one year.

The terrain is mostly hilly and undulating with an altitude ranging from about 79.2 m above mean sea level (msl) to 617.8 m (Venu hill). The hills run in all direction and have moderate slopes. The main hills are Venu, Abhapara, Dantaro, Nangaru, Motohadiyo, Nanohadiyo, Salang, Katal, Kanmero, Mundo, Boliyo, Nanokalo, Motokalo, Dhoro, Karwal and Lambo etc.

The climate of the sanctuary is tropical and semiarid. The duration of rainfall is short during monsoon and rainy days are few. The area is drought-prone and variation in rainfall from year to year is considerable. The average annual rainfall of the area is 697.8 mm. Winter is cool and dry. During January, which is the coldest month, temperature falls to 11.5 °C. May is usually the hottest month of the year. Hot days generally occur in the months of May–June when temperature rises to 47.2 °C.

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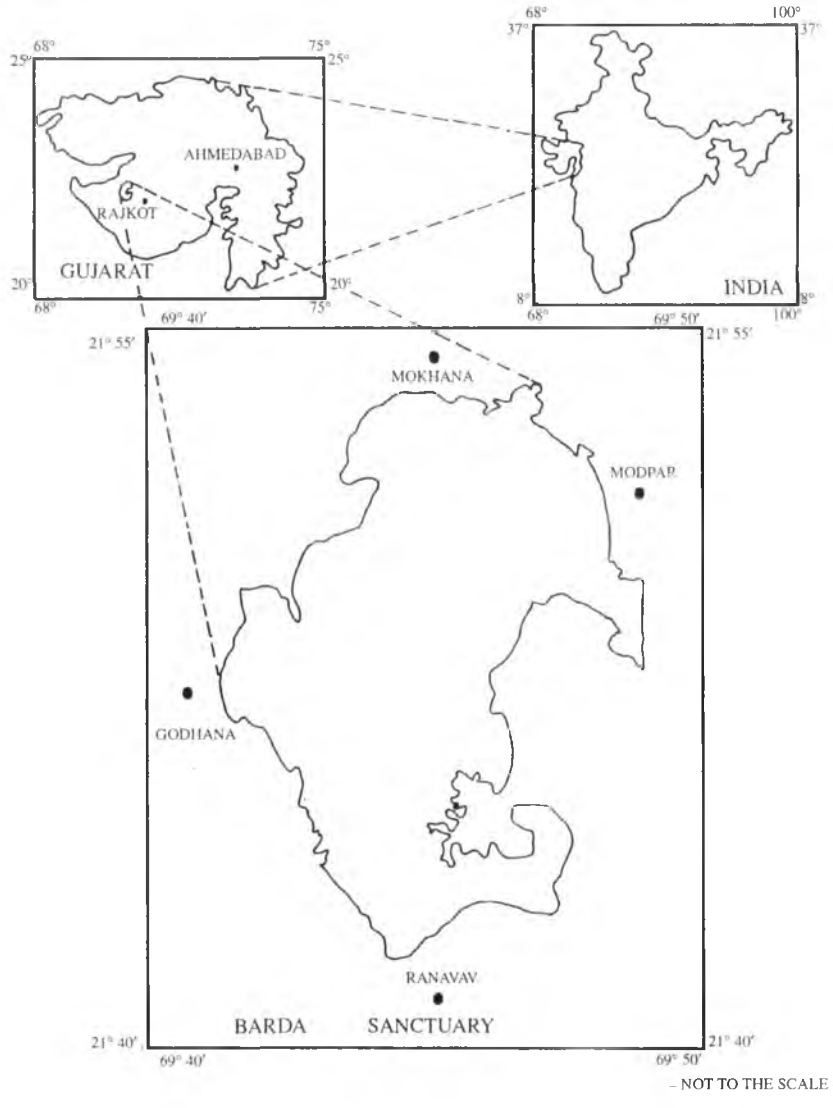

FIGURE 1. Location map showing Barda Sanctuary

Singh *et al.* (2002) recorded 759 plant species (515 indigenous and 244 introduced species) belong to 430 genera of 106 families from the sanctuary, which includes 133 species of tree, one species of liana, 102 species of shrub, 64 species of under shrub, 459 species of herb and climber. The floristic diversity is found to be more on riverain region followed by foothills, slopes, plains and declining towards the peak. The dominant plant species of the sanctuary are *Acacia senegal, Acacia nilotica* var. *nilotica, Dicrostachys cinera, Tectona grandis, Manilkara hexandra, Premna*

herbacea, Gardinia resinifera, Zizyphus xylopyrus, Terminalia crenulata, Cordia gharaf, Lantana camara var. *aculeate* and *Hemidesmus indicus*. Bamboo is observed in scattered patches. As per remote sensing study carried out in 1997 by Gujarat Ecological Education Research (GEER) Foundation, 16.3% of the area was under dense forest, 29.9% under open forest and rest was under degraded category.

Methodology

The study was carried out from August 1999 to July 2000. Butterflies were collected from each vegetation types of the sanctuary. For collection of specimens net were used. Collected specimens were narcotised adding menthol crystals in the bottle and then air dried for the identification. All the specimens were examined carefully and identified using various references such as Talbot (1939, 1947); Wynter-Blyth (1957), and Gay *et al.* (1992). All the scientific names follow Varshney (1979, 1985, 1990) and classification and common English name is after Wynter-Blyth (1957).

A survey of the butterflies of the sanctuary, transects (500 m × 10 m) were laid by using of a pedometer. During monsoon, winter and summer 100, 70 and 100 transects were laid respectively. The status of various butterflies in the sanctuary was scored using presence-absence scoring method in transect and then percentage of occurrence was calculated to determine the status. The score classes used were 1–3% = Rare, 4–6% = Uncommon, 7–18% = Common, >18% = Abundant.

RESULTS AND DISCUSSION

During the study, 59 species and two additional female forms of butterflies were recorded from the sanctuary (Table 1). They belonged to eight families. The family Pieridae contained maximum number of species (17) compared to Acraeidae which contained just one species (Table 2). The number of species recorded with remaining families as follows.

The family Danaidae was represented by four species of which two are rare in the sanctuary. The Plain Tiger (*Danaus chrysippus*) which is one of India's most wide spread and well-known plain butterflies was very common in the sanctuary. whereas the Blue Tiger (*Danaus limniace leopardus*), the Common Tiger or striped Tiger (*Danaus genutia*) and the Common Indian Crow (*Euploea core*) were rare and uncommon respectively.

The family Nymphalidae was represented by 13 species, of which three were abundant; five common, two uncommon and three rare in the sanctuary. The Common Leopard (*Phalanta phalantha*) and the Lemon Pansy (*Precis lemonias*) were found frequent on the damp patches. The Angled Castor (*Ariadne ariadne*), Yellow Pansy (*Precis hierta*) and the Baronet (*Symphaedra nais*) were also more common than other members, while number of Baronet is more at 'Satvirda nes'.

The family Lycaenidae was represented by 14 species, of which four were common, nine uncommon and one rare in the sanctuary. The African Babul Blue (*Syntarucus jesous gramra*) and the Lime Blue (*Chilades laius*) prefer damp patches, while the

Common Pierrot (*Castalius rosimon*) comes to damp patches during the dry season or exceptionally in hot days.

TABLE 1: Butterflies diversity and its status in Barda Wildlife Sanctuary

Sr. No.	common Name	Scientific Name	Season of occurrence	Status
	Danaidae			
1	Blue Tiger	*Danaus limniace leopardus* (Butler)	M	Rare
2	Plain Tiger	*Danaus chrysippus* (Linnaeus)	W, M, S	Common
3	Striped or Common Tiger	*Danaus genutia* (Cramer)	M	Rare
4	Common Indian Crow	*Euploea core* (Cramer)	M, W	Uncommon
	Satyridae			
5	Common Threering	*Ypthima asterope mahratta* Moore	W, M, S	Common
6	Common Evening Brown	*Melanitis leda ismene* (Cramer)	M	Uncommon
	Nymphalidae			
7	Black Rajah	*Charaxes fabius cerynthus* Fruhstorfer	W, M	Rare
8	Common Nawab	*Polyura athamas* (Drury)	W, M	Uncommon
9	Baronet	*Symphaedra nais* (Forster)	W, M, S	Abundant
10	Great Eggfly	*Hypolimnas* (Linnaeus)	M	Rare
11	Danaid Eggfly	*Hypolimnas misippus* (Linnaeus)	W, M	Common
12	Yellow Pansy	*Precis hierta* (Fabricius)	W, M	Abundant
13	Blue Pansy	*Precis orithya* (L.)	W, M	Common
14	Lemon Pancy	*Precis lemonias* (Linnaeus)	W, M, S	Common
15	Peacock Pansy	*Precis almanac* (Linnaeus)	M	Rare
16	Painted Lady	*Cynthia cardui* (L.)	W, M	Common
17	Common Leopard	*Phalantha phalantha* (Drury)	W, M	Uncommon
18	Joker	*Byblia ilithyia* (Drury)	W, M	Common
19	Angled Castor	*Ariadne ariadne* (L.)	W, M	Abundant
	Acraeidae			
20	Tawny Coster	*Acraea violae* (Fabricius)	M	Rare
	Lycaenidae			
21	Common Pierrot	*Castalius rosimon* (Fabricius)	W	Common
22	Rounded Pierrot	*Tarucus nara* (Kollar)	W, M	Uncommon
23	Zebra Blue	*Syntarucus plinius* (Fabricius)	W, S	Common
24	African Babul Blue	*Syntarucus jesous gamra* (Lederer)	M	Common
25	Bright Babul Blue	*Azanus ubaldus* (Cramer)	M	Uncommon
26	Lime Blue	*Chilades laius* (Cramer)	W	Uncommon
27	Tiny Grass Blue	*Zizula gaika hylax* (Trimen)	W	Uncommon
28	Gram Blue	*Euchrysops cnejus* (Fabricius)	W, M	Uncommon
29	Plain Cupid	*Euchrysops parrhasius minuta* Evans	W	Uncommon
30	Peablue	*Lampides boeticus* (Linnaeus)	W, M, S	Uncommon
31	Common Silverline	*Spindasis vulcanus*	W, M	Common
32	Scarce Shot Silverline	*Spindasis elima*	W	Rare
33	Common Guava Blue	*Virachola isocrates* (Fabricius)	W	Uncommon
34	Indian Red Flash	*Rapala airbus sorya* (Kollar)	W, M	Uncommon
	Papilionidae			
35	Common Rose	*Atrophaneura aristolochiae* (Fabricius)	W, M, S	Abundant
36	Common Mormon	*Papilio polytes romulus* Cramer	W	Rare
37	Lime Butterfly	*Papilio demoleus* Linnaeus	W, S	Uncommon
38	Tailed Jay	*Graphium agamemnon* (Linnaeus)	W	Rare
	Pieridae			
39	Common Gull	*Cepora nerissa* (Fabricius)	W, M	Common
40	Pioneer	*Anaphaeis aurota* (Fabricius)	W, M, S	Common
41	Whites or Cabbage Whites	*Pieris sp.*	W, M	Rare
42	White Orange Tip	*Ixias marianne* (Cramer)	M	Common

Sr. No.	common Name	Scientific Name	Season of occurrence	Status
43	Yellow Orange Tip	*Ixias pyrene* (Linnaeus)	W, M, S	Common
44	Small Salmon Arab	*Colotis amata* (Fabricius)	M	Common
45	White Arab	*Colotis vestalis* (Butler)	W, M, S	Rare
46	Large Salmon Arab	*Colotis fausta* (Olivier)	W, S	Uncommon
47	Little Orange Tip	*Colotis etrida* (Boisduval)	W	Uncommon
48	Plain Orange Tip	*Colotis eucharis* (Fabricius)	M	Uncommon
49	Crimson Tip	*Colotis danae* (Fabricius)	W, M, S	Common
50	Great Orange Tip	*Hebomoia glaucippe* (Linnaeus)	W, M	Common
51	Common Emigrant	*Catopsilia crocale* (Cramer)	W, M, S	Abundant
52	Lemon Emigrant	*Catopsilia pomona* (Fabricius)	W, M, S	Common
53	Mottled Emigrant	*Catopsilia pyranthe* (Linnaeus)	W, M	Common
54	Spotless Grass Yellow	*Eurema laeta* Boisduval	W, M, S	Uncommon
55	Common Grass Yellow	*Eurema hecabe* (Linnaeus)	W, M, S	Abundant
	HESPERIIDAE			
56	Indian Skipper	*Spialia galba* (Fabricius)	W	Uncommon
57	Common Banded Awl	*Hasora chromus chromus* (Cramer)	M	Rare
58	Straight Swift	*Parnara naso bada* (Moore)	W	Uncommon
59	Dart	*Potanthus sp.* Scudder	W	Rare

Abbreviations: M = monsoon, W = winter, S = summer.

The family Papilionidae was represented by four species, out of them, one was abundant, one uncommon and two rare in the sanctuary. The Common Rose (*Atrophaneura aristolochiae*) is the most common at Ghumali and Abhapara hill, whereas the Common Mormon (*Papilio polytes romulues*) and the Tailed jay (*Graphium Agamemnon*) are sighted only twice during the study period.

The family Pieridae was the largest family in the sanctuary with 17 species, of which two species were abundant, nine common, four uncommon and two rare. The Common Grass Yellow (*Eurema hecabe*) was the most abundant than all butterfly species; it is probably among the top ten of the world's most numerous butterflies. The Small Salmon Arab (*Colotis amata*) and the Plain Orange Tip (*Colotis eucharis*) are found chiefly in the thorn forest, while the Common Gull (*Cepora nerissa*) is mostly seen on mud pudding with other Pierids.

The family Hesperiidae includes only four species, of which two species were uncommon and two rare. The Indian Skipper (*Spialia galba*) is comparatively more common than the other three members.

The number of butterfly species observed in monsoon was 44 (74.58% of total species), which increased up to 47 (79.66%) in winter but decreased to 17 (28.81%) species in summer. However, 14 species (23.72%) were recorded throughout the year (Tables 1 and 3).

Analysis of the occurrence shows that 13 spedies are rare, 20 species are uncommon, 20 species are common and six species are abundant in the sanctuary (Table 2) and additional two female forms *viz.*, *Hypolimnas misippus* form *inaria* and *Danaus chrysippus* form *dorippus* are very rare and rare in India respectively.

The Danaid Eggfly (*Hypolimnas misippus*) and the common pierrot (*Castalius rosimon*) are schedule-I and the Common Nawab (*Polyura athamas*), the Peablue

TABLE 2. Families, genus, species and status of butterflies in the sanctuary

Sl. no.	Family	No. of Genus	No. of. species	% of species	Abundant	Common	Uncommon	Rare
1	Danaidae	2	4	6.780%	—	1	1	2
2	Satyridae	2	2	3.390%	—	1	1	—
3	Nymphalidae	9	13	22.034%	3	**5**	**2**	**3**
4	Acraeidae	1	1	1.695%	—	—	—	1
5	Lycaenidae	11	14	23.728%	—	4	9	1
6	Papilionidae	3	4	6.780%	1	—	1	2
7	Pieridae	8	17	28.813%	2	9	4	2
8	Hesperiidae	4	4	6.780%	—	—	2	2
	Total	40	59	100%	6	20	20	13

TABLE 3. Seasonal variation in butterfly species in the sanctuary

Season	No. of family	No. of genus	No. of species	Percentage of total species
Monsoon	**8**	**31**	**44**	74.58%
Winter	**7**	**36**	**47**	79.66%
Summer	6	13	17	28.81%
Common throughout the year	6	11	14	23.73%

(*Lampides boeticus*) and the Common Gull (*Cepora nerissa*) are schedule II species as per Indian Wildlife Protection Act (1972).

The Rounded Pierrot (*Tarucus nara*), the White Orange Tip (*Ixias Marianne*), the Little Orange Tip (*Colotis etrida*), the Common Silverline (*Spindasis vulcanus*) and the Scarce Shot Silverline (*Spindasis elima*) are endemic in India and Sri Lanka.

ACKNOWLEDGEMENTS

The authors are grateful to the Director, Gujarat Ecology Education and Research (GEER) Foundation, Gandhinagar for financial support and Forest Department, Gujarat State for giving the permission for work in the sanctuary. We are also thankful to Shri Parbatji Odedara (ACF) and other staff of the sanctuary for the accommodation and other facilities and local people who were assisting us during the course of the study.

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(Received in July 2001; revised and accepted in March 2002)

ENTOMON 27(4): 411–413 (2002)
Short Communication No. ent.27409

Occurrence of fungi *Scopulariopsis brevicaulis* (Saccardo) Bainer and *Aspergillus flavus* Link as entomopathogens of banana stem weevil, *Odoiporus longicollis* Oliver (Curculionidae: Coleoptera)

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ABSTRACT: *Scopulariopsis brevicaulis* (Saccardo) Bainer and *Aspergillus flavus* Link (Trichocomaceae: Ascomycota) were isolated as fungal entomopathogens from banana stem weevil, *Odoiporus longicollis* at Tiruchirapalli and Coimbatore areas of Tamil Nadu. Mean natural mortality of the stem weevil due to *A. flavus* varied from 2.22 to 12.24 per cent. The infection level for *S. brevicaulis* under natural condition varied from 1.82 to 7.32 percent. Pathogenicity of these two fungi to the stem weevil was proved under laboratory conditions.

KEYWORDS: *Odoiporus longicollis*, entomopathogenic fungi

Banana stem weevil (BSW) *Odoiporus longicollis* is the most important pest of banana. It seriously affects banana production systems in Tamil Nadu, Kerala, Karnataka, Andhra Pradesh, Maharashtra, Orissa, West Bengal, and North eastern states of India. The BSW causes serious economic damage to the banana crop as its infestation interferes with nutrient transport resulting in slow growth, reduced fruit filling and high susceptibility to wind logging. In India, two fungal pathogens *viz.*, *Fusarium solani* and *Mucor heimalis* have been recorded from field collected populations of the stem weevil (Anitha *et al.*, 2000). A white muscardine fungus that infests the larvae and pupa with 1% infection has been observed in China (Sun Maolin, 1994). Some general predators like Earwigs (two species) and Acarids were recorded on BSW adults and larvae in China (Sun Maolin, 1994). Parasites are yet to be recorded from the stem weevil populations. Hence, attempts were made to explore the possibility of the occurrence of natural enemies including fungal pathogens and their subsequent use as biocontrol agents in the integrated Pest Management of *O. longicollis*.

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Periodical surveys were undertaken in banana growing tracts around Tiruchirapalli, Coimbatore, Kanyakumari, and Theni districts in Tamil Nadu during 2000–01. Adult weevils and different life stages of *O. longicollis* collected from the banana gardens were maintained separately in the laboratory. They were observed for the emergence of any parasites and mortality due to pathogens. Mortality of adult weevils due to two different types of fungal infections was observed. The pathogens found developing on the adult weevils were isolated and identified. Total number of healthy and infected weevils was counted and percent infection was calculated.

Weevils collected from the cultivars viz., Nendran (French Plantain–AAB) and Myndoli (Giant Plantain–AAB) cultivated in banana gardens at Marudhur near Tiruchirapalli revealed the presence of dark green fungal infection. The infected adults were immobilised and found attached to the leaf sheath with their bodies covered completely by the growth of fungal mycelium bearing abundant dark green spores. The mean natural mortality of adults was 6.92% with a range of 2.22% to 12.24% during the survey period. The associated fungi were isolated and cultured on Potato Dextrose Agar (PDA) medium. In the culture medium, the fungus produced hayaline and septate hyphae. The conidiophores are colourless and terminate into round vesicles which are covered by flask shaped uniseriate or biseriate phialides. The colonies were powdery in texture exhibiting bright green colour. The reverse is yellow–green goldish to red brown. The fungus was identified as *Aspergillus flavus* Link (ITCC Ref. No. 4489-2001). Pathogenicity of the fungus to healthy adults was tested adopting immersion method (Goettel and Inglis, 1997). The weevils were dipped in a fungal suspension of 7×10^6 spores/ml for 5 seconds. The treated weevils were maintained on Nendran pseudostem bits in the rearing containers developed for BSW. Mean mortality of adult weevils was 32.82 and 52.97% at 4 and 8 days after treatment, respectively. The ascomycete fungi, *Aspergillus* is known to produce many carcinogenic toxins (Pierre, 1985) and infect vertebrates. As *A. flavus* is likely to produce health hazards to people engaged in plant protection, its use as a biocontrol agent has severe limitation.

Collections from the cultivar, Pisang Awak (ABB) from the banana gardens at Vedapatti near Coimbatore revealed the presence of adult weevils infected with a fungal pathogen. The mean natural mortality of adult was 3.44% with a range of 1.82 to 7.32%. In the culture, the fungus produced colonies which were moderately rapid growing, granular to powdery, initially white, becoming light brown to buff tan, and form cylindrical annellides and chains of 1-celled, rough-walled conidia having truncate base. It was identified as *Scopulariopsis brevicaulis* (Saccardo) Bainer (ITCC Ref. 4489-2001). Pathogenicity of this fungus was tested with a suspension of 3×10^6 spores/ml for 5 s and the mean mortality of weevils after 4 and 8 days 20.32% to 30.43%, respectively. Mortality caused by *S. brevicaulis* was minimum when compared to *A. flavus*.

The fungi, *S. brevicaulis* and *A. flavus* are hitherto not reported on the BSW, *O. longicollis*. Further studies are currently undertaken to explore the potential use of *S. brevicaulis* as a mycoinsecticide in Banana IPM programme.

ACKNOWLEDGEMENTS

The authors are thankful to Dr. P. N. Chowdhry, ITCC, IARI, New Delhi for identifying the fungi. The authors are grateful to Dr. S. Sathiamoorthy, Director, NRCB and Dr. P. Sundararaju, Head, Division of Crop Protection, NRCB for providing necessary facilities to carry out the research.

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(Received in August 2001; revised and accepted in November 2001)

ENTOMON 27(4): 415–418 (2002)
Short Communication No. ent.27410

Incidence of Pink Bollworm *Pectinophora gossypiella* Saunders on winter and summer cotton in Coimbatore, Tamil Nadu

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ABSTRACT: The activity of cotton Pink Bollworm (PBW), *Pectinophora gossypiella* was minimum in the winter crop (November–February) and its buildup was higher in the summer crop (February–June). Maximum temperature and rainfall were positively correlated with PBW incidence on squares and flowers of both the varieties, however the correlation was negative on bolls. The incidence on squares, flowers and bolls was positively correlated with minimum temperature and negatively correlated with relative humidity.

KEYWORDS: *Pectinophora gossypiella*, cotton

Pink Bollworm (PBW), *Pectinophora gossypiella* Saunders is one of the most damaging insects of cotton worldwide (Curl and White, 1952) and the pest was recorded from nearly all the cotton growing countries of the world (CAB Institute of Entomology, 1990). In India *P. gossypiella* has become a serious pest of cotton causing heavy economic loss by damaging buds, flowers and bolls, however seeds are the main source of food. A wide range in sowing dates of cotton provides continuous food supply throughout the year, which in turn helps the multiplication of the pest. The extend of damage and peak period of incidence vary from year to year depending on seasonal variation. Information on the occurrence of a pest in relation to different seasons is one of the prerequisities for formulating effective and economic control strategies. Investigations were carried out to study the levels of incidence and damage of PBW in winter and summer crops of cotton.

Experiments were conducted in the winter (August–March) and summer crops (February–July) of the year 1994–95, on varities LRA5166 and MCU-5 at Central Institute for Cotton Research, Regional Station, Coimbatore. The crop was sown in rows 75 cms apart and the plant distance within a row was 30 cms. The incidence of PBW was recorded at weekly intervals on squares, flowers and bolls. Squares and bolls of uniform size, 25 numbers each, were collected randomly from the field and

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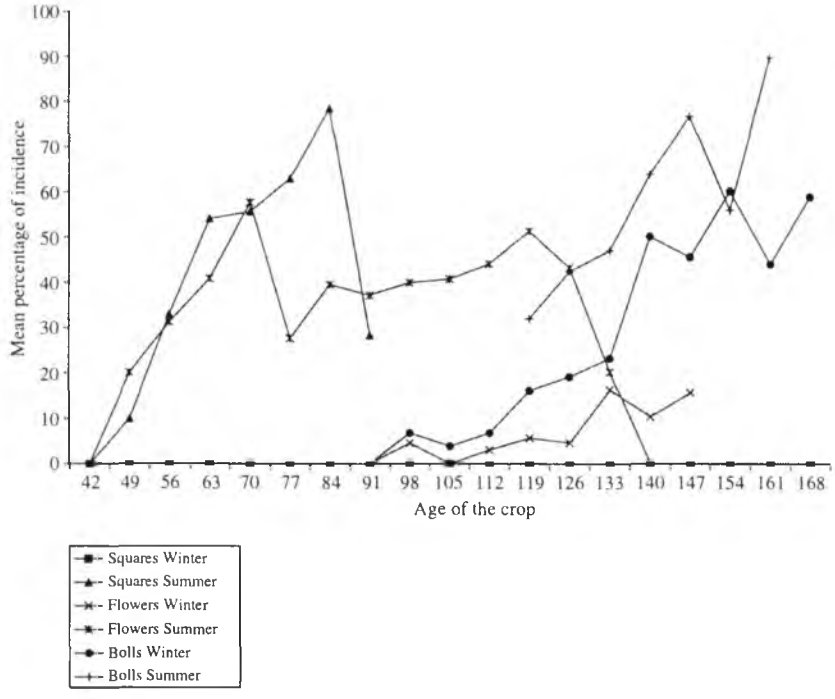

FIGURE 1. Mean percentage of incidence of Pink Bollworm on variety of MCU-5 during winter and summer cotton.

brought to the laboratory and the incidence was recorded by dissecting the squares and bolls. Total number of flowers and those infested by PBW (rosetted) were counted from 100 plants selected randomly.

The PBW incidence on squares was not observed during the winter crop at any growth stage of the crop, on both the varieties. The flowers of the winter crop were less affected and the incidence started 98 days after sowing (DAS) (Fig. 1). The percentage of infestation ranged between 3.03%–16.26% in MCU-5 (Fig. 1) and 1.04%–17.85% in LRA 5166 (Fig. 2). Maximum incidence was recorded 133 DAS. The incidence on bolls started 98 DAS and gradual builup was recorded upto 133 DAS after which a maximum incidence of 60.02% on 154 DAS and 59.01% on 140 DAS was recorded in MCU-5 (Fig. 1) and LRA 5166 (Fig. 2) respectively.

The summer crop recorded a high percentage of infestaion of PBW on sqaures, flowers and bolls. A maximum infestation of 78.46% and 75.38% on squares was recorded 84 DAS in MCU-5 (Fig. 1) and LRA 5166 (Fig. 2) respectively. The flowers recorded a maximum percentage infestation on both the varieties on 70 DAS though there was a reduction in the incidence level at 77 DAS it increased steadily and the pest activity was noticed upto 126 DAS. On bolls the incidence started 119 DAS and the pest was active throughout the remaining season. The early attack on squares by

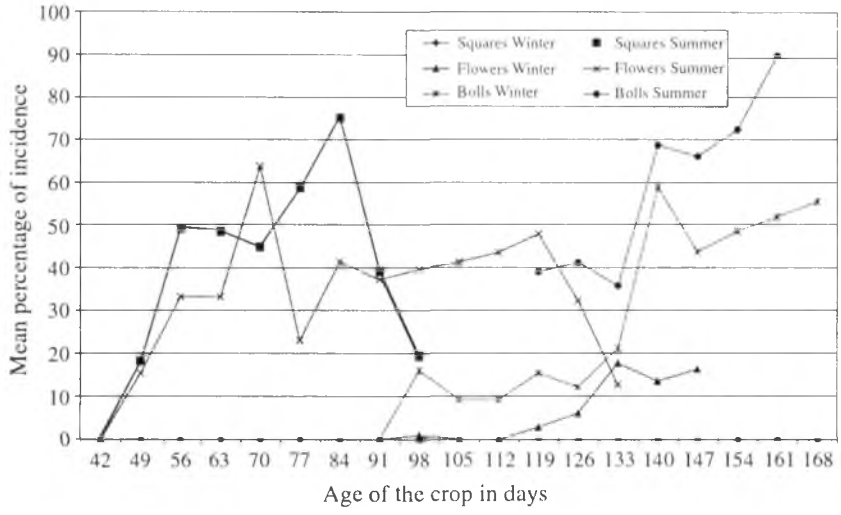

FIGURE 2. Mean percentage incidence of PBW on variety of LRA 5166 during winter and summer cotton.

PBW in summer crop may be due to the immediate availablility of squares for egg laying off the adults emerging from winter crop and also as per the studies by Menon (1979) the squares are the preferred site for oviposition of PBW. The domination of *Helicoverpa armigera* Hubner in the beginning of the winter crop, could be the reason for the low incidence of PBW from November–December. Incidence of the PBW on flowers was higher during the early part of the crop season, incidence on greenbolls prolonged upto the end of the crop season, since seeds are the main source of food. These results are supported by Hussain and Khan (1940), Kittock and Pinkas (1971) and Shaaban and Radwan (1974), who recorded a higher incidence of PBW on squares and flowers as long as bolls are not formed.

The PBW incidence during winter crop was low when compared to summer crop. The study shows that in both the seasons more than 50 per cent of the incidence was noticed after 140 DAS. Though, the incidence started late in the summer crop on bolls (117 DAS) the buildup was fast and the level of incidence was high in summer crop. In winter crop the pest incidence started during the month of December, but the level was very low and similar observations were recorded by Sundaramurthy *et al.* (1987). In summer crop the incidence was high during April and May and this is in accordance with the observations of Surulivelu (1991).

As per Lukefahr and Griffin, 1956 a higer population of PBW moths and larval damage late in the season may be attributed to the reason that the female moth must have fed on bolls during the larval stage and the adults must have food source. As per the present studies the adults emerged during the end of the main crop season (February–March) were found to be fed on bolls and also the adults which emerged during the months of Feb–March had the nector of flowers of February sown summer

TABLE 1. Correlation matrix of PBW incidence with abiotic factors

Abiotic factors	MCU-5			LRA		
	Squares	Flowers	Bolls	Squares	Flowers	Bolls
Max. Temp	0.441	0.081	−0.036	0.475	0.150	−0.071
Min. Temp	0.568	0.735	0.065	0.583	0.749	0.086
Relative Humidity	−0.116	−0.473	−0.417	−0.141	−0.465	−0.470
Rain	0.926	0.599	−0.524	0.895	0.675	−0.546

crop. Also the squares and flowers were not available for the adults emerged from the summer crop during May–June. Probably this may be the reason for the low incidence of PBW during winter and high incidence during summer.

Maximum temperature and rainfall were positively correlated with PBW incidence on squares and flowers of both the varieties, however it was negatively correlated with incidence on bolls. The incidence on squares, flowers and bolls were positively correlated with minimum temperature and negatively correlated with relative humidity respectively (Table 1).

ACKNOWLEDGEMENT

The authors are thankful to Dr. K. Venugopal, Project Co-ordinator and Head, CICR, Regional station, Coimbatore for the facilities extended to the study.

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(Received in August 2001; revised and accepted in December 2001)

ENTOMON 27(4): 419–421 (2002)
Short Communication No. ent.27411

A new species of the genus *Centistes* Haliday (Hymenoptera: Braconidae) from India

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ABSTRACT: *Centistes indicus* sp. nov. is described and illustrated. The genus *Centistes* Haliday is recorded for the first time from India.

KEYWORDS: Hymenoptera, Braconidae, Euphorinae, *Centistes*, new species.

INTRODUCTION

The Genus *Centistes* belongs to the tribe Centistini of the subfamily Euphorinae. The genus was first described by Haliday (1835) as a subgenus of *Leiophron* to accommodate the type species *Leiophron (Ancylus) cuspidatus* Haliday. This genus can be easily distinguished for having extremely short, broad and densely setose ovipositor sheath. So far, about 14 species are known from different parts of the world (Shenefelt, 1969). The genus is reported for the first time from India. The terminology followed is after Achterberg (1993).

Female

Length: 1.685 mm

Head (Figs 1–3)

Head sparsely setose, 1.34× as wide as thorax at tegulae; Antennae 20 segmented, longer than body, scape 2.3× as long as wide, pedicel about as long as wide, F_1–F_{10} about 2× as long as wide, F_{11}–F_{16} about 1.8× as long as wide, F_{17}–1.7× as long as wide, terminal segment pointed apically and 2.6× as long as wide; eyes bare, parallel and not converging posteriorly in frontal view, shortest distance between eyes 1.5× the width of clypeus, frons and some parts of face reticulate rugulose; malar space 0.33× as long as eye length and about as long as basal width of mandible; maxillary palpi 5 segmented; labial palpi 3 segmented; width of antennal socket subequal to malar space; ocelli large arranged in obtuse triangle;

OOL : POL : AOL : ØOD = 7 : 5, 5 : 3, 5 : 5; temple convex, about as long as eye in dorsal view; occipital carina strong and complete.

Mesosoma

Sparsely setose; pronotum smooth, sides of pronotum transversely rugulose anteriorly; mesonotum smooth, notauli absent, scutellar furrow obliquely crenulate, posterior edge of scutellum with few longitudinal carinae; metanotum irregularly longitudinally costate; propodium areolate, clearly subdivided into anterior and posterior halves by a strong transverse carina, surface smooth.

Wings (Figs 4 and 5)

Forewing as long as body, nearly 2.5× as long as wide stigma, 3× as long as wide, marginal cell 3.28× as wide as long, 2-R1 0.23× as long as 1-R1, SRI + 3-SR almost straight, 1-Cu1 0.3× as long as 2-Cu1, m-cu 0.46× as long as 2-SR and interstitial, 2A + 3A present. Hindwing 4.5× as long as wide with 3 sinuate hamuli.

Legs (Fig. 6)

Coxa and femur of hing leg almost smooth, hind coxa 1.27× as long as wide, hind femur 5× as long as maximum width, hind tibia 10× as long as maximum width; hind tibial spur 0.48× as long as hind basitarsus, ratio of hind tarsomeres from basitarsus to tellotarsus = 12 : 6 : 5 : 4 : 7, tarsa, claw simple.

Metasoma (Figs 7 and 8):

First tergite (Fig. 7) basally 0.5× as wide as apical width and about 1.37× as long as apical width, spiracle of first tergite situated on lateral margin, slightly anterior to the middle, first tergite finely rugulose at posterior half; rest of the metasoma smooth and shiny; ovipositor sheath short and broad, densely setose, rounded at apex; ovipositor (Fig. 8) smooth sabre-like with an apical notch.

Colour

Yellowish except: F_1 and F_2 yellowish brown and rest of the antennal segments light brown, stemmaticum and tips of mandibles reddish brown, ocelli black brown, hind tibia apically light brown, wings hyaline, venation brown, metasoma brownish apart from the antero-lateral sides of second and third terga and petiole which are yellowish.

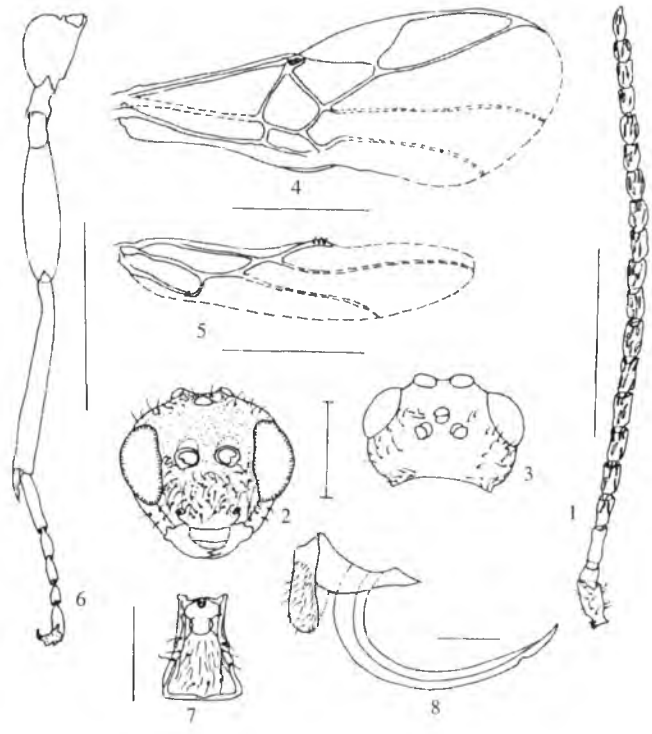
Male

Unknown

Host

Unknown

Holotype ♀, India: Uttar Pradesh, Aligarh; 15, iv, 1969; on wing (Shujauddin).



FIGURES 1–8. 1. Antennae; 2. Head, frontal aspect; 3. Head, dorsal aspect; 4. Fore wing; 5. Hind wing; 6. Hing leg; 7. First metasomal tergite; 8. Ovipositor. Scale lines for Figs 1, 4, 5 and 6 = 0.5 mm, Figs 2, 3 and 7 = 0.25 mm; Fig. 8 = 0.1 mm.

Remarks:

Centistes indicus sp. nov. is closely related to *C. claripennis* (Ashmead) but can be easily separated for having: Propodeum clearly subdivided into anterior and posterior halves by a strong transverse carina, forewing vein 2-R1 0.23× of vein 1-R1 and frons and some parts of face finely reticulate rugulose.

Type material deposited in Zoological Museum, Aligarh Muslim University, Aligarh, Catalogue HB 1023.

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(Received in August 2001; revised and accepted in January 2002)

ENTOMON 27(4): 423–424 (2002)
Short Communication No. ent.27412

Biological suppression of spherical mealybug *Nipaecoccus viridis* (Newstead) (Hemiptera, Pseudoccidae) on acid lime in India

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ABSTRACT: A field experiment was conducted with the Australian ladybird beetle *Cryptolaemus montrouzieri* Muls. for the suppression of spherical mealybug *Nipaecoccus viridis* (Newstead) on acid lime. The results indicated that the mealybug population had declined from 221.30 in March 94 to 1.40 in June 94. The decline in the mealybug population was attributed to the activity of biotic agents *Anagyrus spp.* and *C. montrouzieri*.

KEYWORDS: Spherical mealybug, *Nipaecoccus viridis*, acid lime, *Cryptolaemus montrouzieri*, biological control

The spherical mealybug, *Nipaecoccus viridis* (Newstead) was observed in severe form on four year old acid lime (*Citrus aurantifolia* Swingle) in March 1994 at IIHR Farm, Bangalore, and was very difficult to get controlled with conventional insecticides. The present investigation was carried out to evaluate the efficacy of the Australian ladybird beetle *Cryptolaemus montrouzieri* Muls. against *N. viridis*. The predatory beetle was bred on mealybug infested pumpkins in the laboratory, and a total of 500 predator @ 30/tree was released on 50 acid lime plants infested with mealybugs in March 1994. Subsequent to the release, the population of mealybugs, *C. montrouzieri* and other natural enemies if any were observed at about 15 days intervals on 10 randomly selected infested trees. Four shoots of 30 cm length were removed from each tree and brought to the laboratory. After counting the live mealybugs and predators, the samples were kept over pumpkins in wooden cages to record the emergence of parasitoids and predators. During the study period, the mealybugs were found attacked by *C. montrouzieri* and the encyrtids *Anagyrus agraenis* Saraswat, *A. dactylopii* (How) and *A. mirzai* Agarwal.

The results indicated that the mealybug population had declined from 221.30 on 16th March to 1.40 on 10th June. A maximum population of 8.10 and 10.60 of *Anagyrus* spp. and *C. montrouzieri* was observed in the second week of March '94.

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TABLE 1. Population of spherical mealybug, *Nipaecoccus viridis* and its natural enemies on acid lime

Date of observation	Population/plant (4 shoots/plant) (Mean + SD)		
	Mealybug	*Anagyrus* spp.	*C. montrouzieri*
2-3-1994	148.8 ± 22.16	5.0 ± 3.14	0.0 ± 0.00
16-3-1994	221.3 ± 13.15	8.1 ± 2.75	10.6 ± 3.12
4-4-1994	94.2 ± 8.24	7.4 ± 1.14	7.3 ± 1.30
15-4-1994	68.6 ± 3.16	3.3 ± 0.42	4.2 ± 2.14
2-5-1994	34.4 ± 2.30	1.6 ± 0.70	2.4 ± 0.65
16-5-1994	3.5 ± 0.82	0.0 ± 0.00	3.7 ± 1.14
10-6-1994	1.4 ± 0.21	0.8 ± 0.14	1.3 ± 0.50

SD = Standard deviation.

The decline in the mealybug population was attributed to the activity of the biotic agents (*Anagyrus* spp. and *C. montrouzieri*), since the weather parameters maximum and minimum temperatures, relative humidity and rainfall did not show any significant relationship with the spherical mealybug population. The effectiveness of *C. montrouzieri* against *N. viridis* infesting citrus and *Erythrina glauca* (Tirumala Rao and David, 1958) and ber (Mani, 1993) had been reported earlier.

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(Received in March 2002; revised and accepted in June 2002)

ENTOMON 27(4): 425–429 (2002)
Short Communication No. ent.27413

Biological control of uzifly, *Blepharipa zebina* Walker, (Diptera: Tachinidae) infesting tasar silkworm, *Antheraea mylitta* Drury

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ABSTRACT: The present study reports the efficiency of four indigenous parasitoids of uzifly infesting tasar silkworm, namely *Nesolynx thymus* (Girault) (Eulophidae) *Brachymeria lasus* Walker (Chalcididae), *Thrichopria* sp (Diapriidae), and *Exoristobia philippiensis* (Ashmead) (Encyrtidae). The percentage of uzifly parasitized by *N. thymus* was maximum while parasitization by other three species was less. Host searching ability was also high in *N. thymus* compared to other species.

KEYWORDS: *Antheraea mylitta*, endoparasitoid, *Blepharia zebina*, biological control, hyperparasitoids

INTRODUCTION

The tachinid fly, *Blepharipa zebina* Walker is a larval endoparasitoid of tasar silkworm. *Antheraea mylitta* Drury (Lepidoptera: Saturniidae) and causes considerable damage to tasar silk industry (Jolly *et al.*, 1974; Singh and Thangavelu, 1991). Sometimes, the loss due to this fly is about 40% in tropical tasar zone (Chaturvedi *et al.*, 1986; Singh and Thangavelu, 1991). In recent years substantial efforts were made towards biological control of uzifly, *B. zebina* (Singh *et al.*, 1995; Kishore *et al.*, 1996). Several natural enemies including four parasitoids of uzifly were identified. The information emanating from such studies assumes importance especially because the secondary parasitoids are known to act as mortality factors of primary parasitoids (Kumar *et al.*, 1993).

The investigations reported here provide some insight into the degree of efficiency of four indigenous parasitoids of uzifly, earlier reported by various workers in the recent years (Singh *et al.*, 1995; Kishore *et al.*, 1996; Kumar *et al.*, 1991). The rate of parasitisation and host searching ability of each of the parasitoid under identical

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TABLE 1. Parasitisation potential, adult emergence per parasitised pupa and searching ability of parasitoids of uzifly

Sl No.	Natural enemies used in study	% of uzifly puparia parasitised by parasitoids	Emergence of parasitoids per parasitised pupa	Searching ability (in meter)
1	*Nesolynx thymus*	40.80 ± 3.49	75.88 ± 10.63	65
2	*Trichopria* sp	20.20 ± 1.92	59.28 ± 13.47	30
3	*Exoristobia philippinesis*	15.40 ± 1.82	30.44 ± 9.86	65
4	*Brachymeria lasus*	4.60 ± 1.92	1.0 ± 0.00	30
	CD at%	3.22	15.76	—

condition were determined to serve as a biocontrol agent for the control of uzifly, *B. zebina*.

The indigenous hymenopteran species used in this study are *Nesolynx thymus* (Girault) (Eulophidae), *Brachymeria lasus* Walker (Chalcididae), *Trichopria* sp. (Diapriidae) and *Exoristobia philippinensis* (Ashmead) (Encyrtidae) (Singh *et al.*, 1995; Kishore *et al.*, 1996, 1999). Mass multiplication of these parasitoids was carried out in an insectary at 26 ± 2 °C and 65.5 ± 5% RH. Adults of all these parasitoids for mass multiplication were obtained from the laboratory colony maintained using the methods proposed by Kumar (1987). House fly, *Musca domestica* puparia served as host for mass multiplication of these parasitoids. House fly puparia were collected from labortory colony maintained for the purpose. Healthy puparia of *M. domestica* were placed in glass cages and allowed to be parasitised separately by *N. thymus, B. lasus, Trichopria* sp. and *E. philippinensis*. A ratio of 1:10 was maintained between female parasitoid and host puparia to get maximum number of parasitoids. Adult parasitoids were collected and fed on 50% agueous honey solution for 1–2 days before being transported for field release. Release of parasitoids was done in the tasar silkworm rearing fields and grainages (egg production unit) at different sites. A ratio of 1:2 was maintained between parasitoid female and uzifly puparia. Release of parasitoids was done three times at an interval of 15 days and host puparia were kept in wire mesh cages (100 each cage) in rearing field and grainages and were replaced once in a week with a new set of puparia. Ten replications were maintained for collection of data. Exposed uzifly puparia were collected back from the field and were subsequently cultured in the laboratory for recording of data on parasitisation potential, emergence of adult parasitoid per parasitised pupa and on host searching ability of the parasitoids. The experiment was carried out at 25 ± 1 °C and 65 ± 5% RH. Accured data were subjected to statistical analysis (Fisher and Yates, 1963) to determine the level of significance on the variations recorded on the parameters mentioned above.

The percentage of uzifly, *B. zebina* puparia (40.80 ± 3.49%) parasitised by *N. thymus* was maximum (Table 1) while the degree of parasitisation by *Trichopria* sp., *E. philippinensis* and *B. lasus* recorded were 20.20±1.92%, 15.40±1.82% and 4.60±

TABLE 2. Percentage of parasitisation by *N. thymus* at weekly intervals after Field release

Week after release	Percentage of parasitisation ± SD	
	Silkworm rearing field	Grainage
I	41.47 ± 22.19	75.07 ± 9.04
II	21.02 ± 5.86	29.67 ± 6.83
III	12.50 ± 19.09	6.35 ± 1.49

1.95% respectively. Even the number of *N. thymus* adults (75.88 ± 10.63) developed successfully in one parasitised uzifly pupa was significantly higher compared to the number of adults of *Trichopria* sp. (59.28±13.47), *E. philippinensis* (30.44±9.86) and *B. lasus* (1.0 ± 00) emerged out from each parasitised host puparium. Host searching ability is the most desirable characteristic of an efficient natural enemy. When *N. thymus, E. philippinensis, Truchopria* sp. and *B. lasus* were released periodically at different distances of silkworm rearing fields and grainages at Central Tasar Research and Training Institute, (CT&TI), Ranchi (India), it was found that the former two parasitoids were capable of searching the host puparia located even upto 65 m as against 30 m, in the case of other two parasitoids. The range of parasitisation by each parasitoid and the maximum distance of searching ability and parasitisation potential vary among the four parasitoids studied (Table 1).

The parasitisation was not shared by any of the hymenopteran parasitoid species. It, therefore, appears that either all the four parasitoids discriminate the uzi puparia parasitised by the other parasitoids species, or the parasitoid species which attacked the uzi puparia first seems to complete the development successfully. These factors have also been attributed to be the reasons for successful development of the hymenopteran parasitoids species when a single host puparia exposed to more than one parasitoid species (Ryan and Medley, 1972).

Further, if simultaneous competition by two or more parasitoid species for one stage of a host takes place, it is the one species which is usually become dominant due to specific fitness (Flanders, 1965) and this dominant species is designated as superior or efficient (Smith, 1929). In the light of this statement, it can therefore, be inferred from the present study, that among four parasitoids, *N. thymus* is the most efficient parasitoid against *B. zebina*, with higher degree of host searching ability and also parasitisation potenital.

In continuation of the above study when *N. thymus* adults were released in tasar silkworm rearing fields and grainages at CTR&TI, Ranchi, the average parasitisation percentage obtained at weekly intervals after release is recorded in Table 2.

The results indicate that to realise maximum parasitisation potential, *N. thymus* should be released at weekly interval.

Sometimes an indigenous or exotic natural enemy is already estabilished but its numbers are insufficient to lower the target organism below the pest status. The natural enemy numbers are then boosted by rearing in large numbers in the laboratory

for regular or occasional release in the field. This method is generally known as augmentation of natural enemies (Ridgway and Vinson, 1977) which is now being tested for biocontrol of uzifly in our present study.

Generally classical biocontrol involves searching for natural enemies in their native area where they exert an important regulatory pressure upon the pest. Such natural enemies are collected and sent to the country or area where the pest population outbreak is frequent. This method is the most well known aspect of biocontrol. It is emphasised here that few parasitoids of uzifly, *B. zebina* are reported from Ranchi (Bihar) and therefore, there is urgent need for search of more parasitoids from other agroclimatic zones where this uzifly is a serious problem for tasar silk industry.

Though eight parasitoids of uzifly have been reported in recent years, (Singh *et al.*, 1995; Kishore *et al.*, 1996) detailed studies have been conducted with regard to four parasitoids only. In the case of others, either their culture has not been maintained or their report is of recent origin and therefore, their biological studies are still incomplete or to be initiated. It is, therefore, felt necessary to set up a germplasm bank of the parasitoids of uzifly in one or two identified institutes, provided these organisations take the responsibility of maintaining the culture and supply the same as and when required by the agencies engaged in evolving biocontrol of uzifly.

ACKNOWLEDGEMENT

Authors are thankful to Suresh Rai, Statistician, Central Tasar Research and Training Institute, Ranchi for analysis of the experimental data.

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(Received in September 2001; revised and accepted in July 2002)

ENTOMON **27(4):** 431–435 (2002)
Short Communication No. ent.27414

Studies on the dose-age-mortality relationship of nuclear polyhedrosis virus of potato cutworm, *Agrotis segetum*

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ABSTRACT: The pathogenicity of nuclear polyhedrosis virus (NPV) of potato cutworm, *Agrotis segetum* was tested in the laboratory to all the six instars through diet surface contamination technique using 3.4×10^7 POBs/ml (polyhedral occlusion bodies). The above study revealed that the mortality appeared on the third day to fifth day after inoculation depending upon the instars. Cent per cent mortality occurred in first and second instars within 6 day after inoculation while more than 80 per cent mortality was achieved during the same period in third and fourth instars larvae. No death occurred in sixth instar larvae. The median dose of the NPV (LD_{50}) of the NPV required for third instar larvae of *A. segetum* for 7th day was found to be 1.06×10^6 POBs.

KEYWORDS: nuclear polyhedrosis virus, *Agrotis segetum*

Agrotis segetum commonly called potato cutworm, is a polyphagous, nocturnal, subterranean insect pest, which attacks many important crops like potato, barley, oats, peas, Bengal gram, cauliflower, cabbage, tobacco, beet-roots etc. This pest is difficult to control with non-persistent chemical insecticides because of the behaviour of older larvae, spending most of their times in the soil and only feeding on the soil surface by night. Further, this pest has started showing resistance to many chemical pesticides including the bacterial toxin of *Bacillus thuringiensis* (Jalali *et al*, 1998). Moreover, in the present day context there is an increasing demand for substituting chemical insecticides with eco-friendly microbial pesticides. Nuclear polyhedrosis virus (NPV) has immense potential for control of *A. segetum* as a microbial pesticide. Though the occurrence of NPV in India on *A. segetum* has been reported by Narayanan (2000), there was no further study. Hence, an attempt has been made to study the pathogenicity of NPV of *A. segetum* and the same has been presented in this paper.

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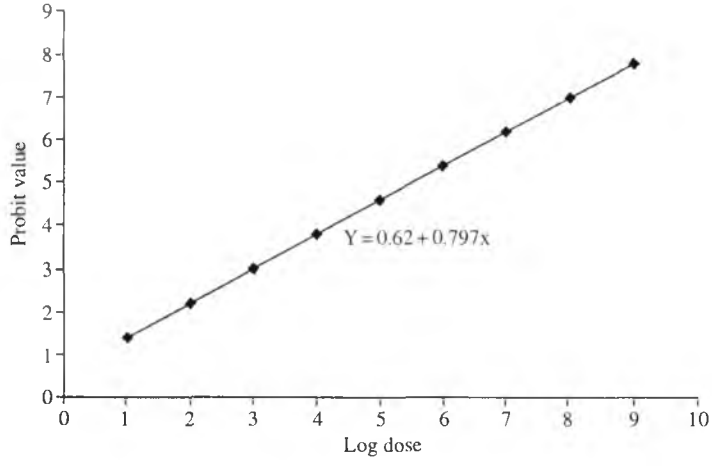

FIGURE 1. Regression line of NPV against *A. segetum* on 7 day.

The polyhedral occlusion bodies (POBs) were extracted from dead larvae of *A. segetum* and purified through differential centrifugation. The final concentration was determined as number of POBs/ml by way of using a Haemocytometer (Neubaur improved double ruling). Equal size *A. segetum* larvae for each instar were selected for the bioassay study. Larvae were starved for 2 hr before allowing them to feed on virus treated diet for better feeding and acquisition of the virus. Two sets of experiments were conducted in the following manner to study the effect of AsNPV on *A. segetum* larvae.

Bioassay studies were conducted with first, second, third, fourth, fifth and sixth instar larvae of *A. segetum* through diet surface contamination technique. One concentration (3.4×10^7 POBs/ml) was used for the study along with a control. The diet surface was smeared with 0.1 ml of the said concentration and dried in shade whereas the control surface was smeared with distilled water. Ten larvae of each instar of equal size in three replications were taken in separate cups for each concentration. The diet surface was treated only once and larvae were allowed to feed *ad libitum*. Mortality counts were taken at an interval of 24 hr and data were statistically analysed.

Bioassay studies were also carried out with third instar *A. segetum* larvae through diet surface contamination technique to study the dose-mortality response. The stock solution of AsNPV was serially diluted and four concentrations viz., 3.4×10^8, 3.4×10^7, 3.4×10^6, 3.4×10^5 POBs/ml along with control was tested. The diet surface was smeared with 0.1 ml of virus solution and with distilled water in case of control and allowed to dry in shade. Treatment was given only once and larvae were allowed to feed continuously till death. The mortality data were recorded daily. The mortality data obtained were subjected to probit analysis (Finney, 1964) for 7th day after inoculation.

The virus was highly virulent and caused mortality of the treated larva in 3–6 days after ingestion of the virus depending upon the stage of the larva. The caterpillar in

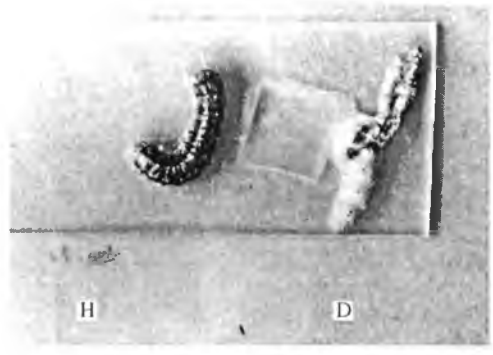

FIGURE 2. NPV infected *A. segetum*. H = Healthy, D = Diseased, note the skin break and oozing out of fluid.

the advanced stage of infection became sluggish and lost appetite. The larvae became lighter in colour, specially on the dorsal surface of the body with reduced size. The diseased larvae sometimes discharge a grayish fluid from their mouth. The integument of the dead larvae became fragile and burst, liberating the liquefied body contents (Fig. 2). It was also observed that treated larvae had longer intermoulting period or less number of moulting Some of the larvae when treated at fifth or sixth instar, though pupated normally, they formed malformed pupa and subsequently died.

It is evident from the Table 1, that the mortality started from third day after treatment in first, second, and third instar larvae. Within six day after treatment first and second instar larva showed cent per cent mortality. In the same time, more than 80 per cent mortality was observed in the case of third and fourth instar larvae. However, mortality in the fifth instar larvae was only 20 per cent, which was significantly lower than other stages. In the fifth instar mortality occurred only fifth day onwards after treatment and no mortality was recorded in sixth instar as well as in control and continued till normal pupation. Response of first and second instar larvae to AsNPV in respect of mortality was on par throughout the period and they showed significantly higher mortality than any of the remaining instars. Hence, study on the susceptibility of different larval instars revealed that the earlier instars were very highly susceptible to the virus. Many earlier investigations of insect host baculoviruses interactions have shown that as host age increases, susceptibility to the virus generally decreases and the time taken for infected larvae to die increases (Rabindra and Subramanian, 1974; Narayanan, 1979; Jayachandran and Chaudhary, 1996). Prasad and Ramakrishnan (1993) experimentally concluded that the virus infectivity in later instar larve were reduced due to certain physiological barriers as that of midgut along with dilution effect of the virus due to the increased mass accompanied with the growth. Most of the published data indicate the decrease in susceptibility is largely due to increasing body weight. In the present investigation, larval body weight ranged from 0.068 mg in case of first instar larva to 238.1 mg in case of sixth instar larva with a mortality range of 100 to 0 per cent. The

TABLE 1. Percentage mortality of AsNPV on *A. segetum*

Instars	Days after treatment			
	3	4	5	6
First	13.33	80.00	86.67	100.00
	(21.14)	(63.93)	(68.85)	(90.00)
Second	20.00	70.00	90.00	100.00
	(26.07)	(57.00)	(75.00)	(90.00)
Third	20.00	30.00	60.00	90.00
	(26.07)	(33.00)	(50.85)	(75.00)
Fourth	0.00	10.00	30.00	80.00
	(0.00)	(15.00)	(33.00)	(63.93)
Fifth	0.00	0.00	20.00	20.00
	(0.00)	(0.00)	(22.14)	(22.14)
'*F*" test	**	**	**	
SEM	2.9409	4.9903	6.5918	
CD at 1%	13.9518	23.6741	31.2714	

** Significant at 1% and 5%.
(Figures in the parentheses are transformed value.)

TABLE 2. LD_{50} of AsNPV on third *A. segetum*.

Days after treatment	LD_{50}	Regression line	Fiducial limit	
			Minimum	Maximum
7	1.06×10^6	$Y = 0.62 + 0.797X$	3.8327	5.9995

Y = Probit value; X = Log concentration; LD_{50} = Lethal dose 50.

apparent resistance of the older larvae could also be due to the fact that they undergo pupation before the virus can exert its influence on the larva.

The dose mortality response data for cutworm, *A. segetum* to its NPV showed that the dosage required to cause 50 per cent mortality varied with time of exposure of the larvae to the virus. The dosage required was found to be lower as the time of exposure increased. Dose has an inverse relationship with exposure period for 50 per cent mortality of test population. The median lethal concentration of the NPV required for 7 days exposure period was found to be 1.06×10^6 POBs (Table 2). Kenchareddi and Jayaramaiah (1997) and Battu and Ramakrishnan (1987) reported that as viral concentration is increased, the time required for 50 per cent mortality was decreased and vice versa. The regression line of probit value and log concentrations for 7 days is shown in Fig. 1 and Table 2. Regression equation is found to be $Y = 0.62 + 0.797X$ and $Y = 11.98 - 1.36X$.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. S. P. Singh, Project Director, Project Directorate of Biological Control, Bangalore for providing the necessary facilities. The research was carried out under the NATP Project on Team of Excellence for Human Resource Development in Biological Control.

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(Received in July 2001; revised and accepted in July 2002)

ENTOMON 27(4): 437–440 (2002)
Short Communication No. ent.27415

Effect of continuous rearing on the biotic potential of *Ceutorhynchus portulacae*, a potential biocontrol agent of *Portulaca oleracea*

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ABSTRACT: The curculionid weevil *Ceutorhynchus portulacae* was identified as a potential biocontrol agent that could be manipulated for suppression of the purslane weed *Portulaca oleracae*. The weevils were mass multiplied under laboratory conditions for augmentative field releases against the target weed. A study was carried out to determine the effect of continuous rearing on the biotic potential of the insects that could help us to rejuvenate the culture for quality maintainance. The results indicated that there is no detoriation in the quality of the biocontrol agents produced, even after rearing them continuously for three years. The present study discusses the factors that could have contributed in maintaining the quality of the bioagents produced.

KEYWORDS: *Portulaca oleracae*, *Ceutorhynchus portulacae*, continuous laboratory rearing

A plant of exotic origin, *Portulaca oleracea* L. is considered as a serious weed of vegetables, vineyards, banana orchards, maize, cotton, groundnut, sorghum, sugarcane, sunflower and rice (Chadha *et al.*, 1995; Mandal, 1990). The curculionid weevil *Ceutorhynchus portulacae* (Fig. 1) was identified as a potential indigenous biocontrol agent that could be mass reared and released for effective suppression of the weed (Ganga Visalakshy and Jayanth, 1997). Mass multiplication of the biological control agents under ambient environmental conditions for timely field releases is an essential pre-requisite for any successful biological control programme. However this has been reported to effect the fertility, fecundity, longevity and searching behaviour of the bioagents resulting in gradual detoriation and reduction in their effectiveness under field conditions (Mackauer, 1981; Hooper *et al.*, 1993). Hence a study was carried out to determine the effect of mass multiplication under laboratory conditions on the

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FIGURE 1. *Ceutorhynchus portulacae adult*, a natural enemy of the weed *P. oleracea*.

biotic potential of *C. portulacae*, which could be useful in determining the time interval needed for rejuvenation of the culture.

Field collected weevils were mass multiplied under laboratory conditions on purslane plants enclosed in large wooden cages as described by Ganga Visalakshy and Krishnan (2001). The cages were of 4 × 3 ft height, with nylon wire mesh on three sides and top and a sliding glass front. Seed pans with a month old purslane plants were kept inside the cage. To this cage, a week old adults at the rate of 5 pairs per cage were released. The cages with the plants and weevils were frequently kept outside, exposed to the climatic conditions. By one week, mines start appearing on the leaves. These were collected once in three days and released into pupation cages, where a twig of purslane was also provided. Adults start emerging after about 5 days. The adults emerged from the pupation jar were released into fresh cage with the purslane plants and the process was repeated.

Comparative studies on the biotic potential (fecundity and sex ratio) of the weevils were made between the wild type and laboratory-reared adults at every 5^{th} generation upto F 30^{th} generation. The culture was maintained continuously in the laboratory and comparison with the wild type was made after three years.

The effect of continuous rearing of *C. portulacae* is presented in Table 1. Studies made up to 30 generations indicated no significant differences in the sex ratio and fecundity of *C. portulacae*. The sex ratio of the wild type (F generation) was recorded as 1 : 1 with 333 eggs per female. The sex ratio of adults of 5 to 30th generation was found between: 1.0 : 0.8 to 1 : 1 (female : male) with a mean fecundity of 325, 305, 301 and 316 eggs for 5^{th}, 10^{th}, 20^{th} and 30^{th} generation respectively. Rearing the weevils continuously for three years was found to cause no detoriation in the above parameters. As average fecundity of 306 eggs per female and 1 : 0.9 sex ratio was recorded in the generation reared up to three years. An average longevity of 91 days

TABLE 1. Effect of continuous laboratory rearing on the functional potential of *C. portulacae*

Treatments	Fecundity/female	Sex ratio (Female : Male)
F (Wild type)	333	1 : 1
F^5	325	1 : 1
F^{10}	305	1 : 0.9
F^{20}	301	1 : 0.8
F^{30}	316	1 : 1
F^n (after three years)	306	1 : 0.9

(varied from 78–123 days) was recorded for the wild type. The adults reared up to three years recorded an average longevity of 89 days, indicating no detoriation in the quality of insects ever after three years of continuous rearing.

Many authors in the field of biological control have opined that continuous rearing of the bio-agents in controlled enclosed environment could be one of the factors causing quality detoriation (Jayanth and Geetha, 1994; Nagarkatti and Nagaraja, 1978). Also the quality of food provided is reported to affect the biotic potential of *Neochetina* spp and *Zygogramma bicolorata*, natural enemies of water hyacinth and Parthernium respectively (Center and Wheeler, 1991; Annadurai, 1990). In our mass rearing programme, the weevils were maintained in large wooden cages that were frequently exposed to the outside climatic conditions. Also the weevils were exposed to plants of less than a month old as our studies have reported that plant phenology was found to affect the survival, longevity and fecundity of the weevils (Ganga Visalakshy, 2000). These factors could have helped in maintaining the quality of *C. portulacae* for a longer time thus preventing the detoriation of the biotic potential of the weevils. The easy mass rearing combined with the possibility of maintaining the culture for a longer period without causing any negative impact on the quality of the bioagents produced adds to the feasibility of manipulating it for effect suppression of the weed, *P. oleracea*.

ACKNOWLEDGEMENT

The senior author is grateful to the Director, Indian Institute of Horticultural Research, Bangalore for sanctioning of the leave for pursuing Ph.D. programme.

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(Received in September 2001; revised and accepted in October 2002)

ENTOMON 27(4): 441–446 (2002)
Short Communication No. ent.27416

Response of *Alphitobius diaperinus* Panzer to *Bacillus thuringiensis* Berliner var. *kurstaki*

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ABSTRACT: LD_{50} values for first to eighth instar larvae of *Alphitobius diaperinus* Panzer feeding on *Bacillus thuringiensis* var. *kurstaki* treated food up to pupation were obtained as 62.773, 72.682, 92.233, 101.913, 114.203, 117.79, 137.662 and 137.936 ppm. More than 50% reduction in pupation and adult emergence were achieved at 150 and 100 ppm doses respectively irrespective of treated larval age. The sex-ratio was not affected by any dose of Btk.

KEYWORDS: Btk, *A. diaperinus*, toxicity, adult emergence

The lesser meal worm, *Alphitobius diaperinus* Panzer, frequently infests the commercial poultry (Cotton, 1963; Hinton and Corbet, 1975). It is also a notorious pest of a great variety of stored grains and cereal products (McAllister *et al.*, 1995). *Alphitobius* species beetle also attack sorghum (Lavigne, 1971), potatoes (Sharma *et al.*, 1987), timbers (Bandeira *et al.*, 1989), and also associated with the sericulture in India (Veer *et al.*, 1990). Life stages of *A. diaperinus* transmit a number of disease organisms, of which *Salmonella* species infects human beings (Geissler and Kosters, 1978).

In Pest Management Programme *Bacillus thuringiensis* Berliner is being used as a biocontrol agent. *B. thuringiensis* var. *kurstaki* (Btk) is a lepidopteran specific bacterium, and gained a reputation in controlling the lepidopteran pests of the stored products (Burges, 1964; Kinsinger and McGaughey, 1976, 1979; McGaughey, 1978; Faruki, 1994). Varieties of *B. thuringiensis* e.g. *B. t.* var. *tenebrionis/sandiego* (Hernstadt *et al.*, 1986; Mummigatti *et al.*, 1994), *B. t.* var. *morrisoni* (Krieg *et al.*, 1984) and EG 2158 (Donovan *et al.*, 1988) were reported to be effective in beetle control.

The stored grains and cereals are very often infested by both the moths and the beetle at the same time. Abdel-Razek *et al.* (1999) found *B. thuringiensis* had negative effects on food consumption and energy use by *Tribolium castaneum* Herbst. Falcon (1971) stated that presence of *B. thuringiensis* in grain stores may play an important

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TABLE 1. LD_{50} values, 95% confidence limits, regression equation and χ^2 value for *A. diaperinus* larvae treated with *Bacillus thuringiensis* var. *kurstaki*

Instars	LD_{50} (ppm)	95% confidence limits		Regression equation	χ^2 value (df)
		Lower	Upper		
1st	62.77288	54.08401	72.85767	$Y = 2.205126 + 1.554632X$	7.170166(3)
2nd	72.68221	58.08275	90.95136	$Y = 1.80014 + 1.719035X$	8.891662(3)
3rd	92.23288	71.28663	119.3339	$Y = 1.62435 + 1.717988X$	12.18728(3)
4th	101.9133	90.57626	114.6693	$Y = 1.78627 + 1.902855X$	7.667328(3)
5th	114.2029	90.13409	144.6988	$Y = 1.301884 + 1.797229X$	10.13623(3)
6th	117.7901	85.83209	161.647	$Y = 1.895181 + 1.49911X$	12.5264(3)
7th	137.6622	108.1895	175.1638	$Y = 1.263176 + 1.747147X$	8.313011(3)
8th	137.9357	101.2675	187.8812	$Y = 1.29256 + 1.809032X$	14.62824(3)

role in integrated pest management system of the stores. The present work is aimed to find out the effects of Btk on *A. diaperinus*.

The source of Btk was Dipel® 1600 IUP/mg, a wettable powder of 32% a.i. Required quantity of Dipel® was mixed with the food (19 : 1 flour and yeast) to prepare different doses.

First to eighth larval instars of *A. diaperinus* were exposed to treated diet of different doses up to pupation. The cumulative larval mortality in treated food was assessed for different larval instars. The percentage mortality was corrected according to Abbott's formula (Abbott, 1925) and the median lethal doses (LD_{50}) were determined for each larval instar. Fifty larvae of each instar were used for each dose. Similar number of larvae of each instar was kept on untreated food as control. All the experiment was replicated thrice.

First to eighth larval instars were exposed to Btk treated diet up to adult emergence. The doses of Btk used were 25, 50, 100, 150, 200 and 300 ppm. One hundred and fifty larvae of each larval instars were used for each dose. A similar number of larvae of each instar were raised on untreated diet as control. The pupal recovery and adult emergence were recorded. Sex of the pupae was determined according to Halstead (1963).

Fresh potato slices were used in both the treated and untreated food of *A. diaperinus* larvae, which was changed everyday. The food was also replaced after every three days. All the experiments were conducted at 30 ± 1 °C without controlling light and humidity.

Pupal recovery of the larvae raised on untreated food varied from 76 to 84.7%. Whereas pupal recovery of the larvae were significantly ($P < 0.001$) reduced in Btk treated diet at each dose and each instar (Table 2). No pupa was obtained from any larval instar feeding on 300 ppm of Btk. The larvae showed tolerance to Btk treatment with the increase of age. Btk at any dose did not affect the sex-ratio of the pupae (Table 2).

TABLE 2. Pupal formation, sex-ratio and adult emergence in *A. diaperinus* treated with Btk

Instar	Doses ppm	Larvae used	Sex-ratio				Adult emergence (%)
			Male (%)	Female (%)	Ratio	*t*-values	
1st	Control	150	42	42	1:1	0	72067
	25	150	29.33	33	0.89:1	0.11	48
	50	150	25.33	22.67	1.12:1	0.47	38067
	100	150	18.67	17.33	1.08:1	0.27	26.67
	150	150	16	10	1.6:1	1.48	16.67
	200	150	7.33	4.67	1.57:1	0.96	4.67
	300	150	0	0	0	0	0
2nd	Control	150	39.33	40.67	0.97:1	0.18	72
	25	150	30.67	30	1.02:1	0.11	50.67
	50	150	26.67	23.33	1.14:1	0.58	40
	100	150	20.67	17.33	1.19:1	0.67	28
	150	150	12.67	13.33	0.95:1	0.16	16.67
	200	150	6.67	5.33	1.25:1	0.48	3.33
	300	150	0	0	0	0	0
3rd	Control	150	37.33	38.67	0.97:1	0.19	68
	25	150	30.67	31.33	0.98:1	0.08	53.33
	50	150	25.33	25.33	1:1	0	40.67
	100	150	22	20	1.1:1	0.38	33.33
	150	150	18	12.67	1.42:1	1.2	18.67
	200	150	10	4.67	2.14:1	1.83	6
	300	150	0	0	0	0	0
4th	Control	150	41.33	36.67	1.13:1	0.65	72
	25	150	34	32	1.06:1	0.3	58
	50	150	26.67	25.33	1.05:1	0.23	44.67
	100	150	19.33	20	1.97:1	0.14	34.67
	150	150	18	13.33	1.35:1	1.03	17.33
	200	150	6.67	9.33	0.71:1	0.83	6.67
	300	150	0	0	0	0	0
5th	Control	150	40	40.67	0.98:1	0.09	68
	25	150	36.67	32.67	1.12:1	0.59	53.33
	50	150	30.67	28	1.10:1	0.43	40.67
	100	150	26.67	24	1.11:1	0.46	33.33
	150	150	16	20.67	0.77:1	0.95	18.67
	200	150	11.33	8.67	1.31:1	0.73	6
	300	150	0	0	0	0	0
6th	Control	150	42	36	1.17:1	0.83	71.33
	25	150	33.33	30.67	1.09:1	0.41	54.67
	50	150	27.33	27.33	1:1	0	46.67
	100	150	26.67	22	1.21:1	0.81	38.67
	150	150	20.67	18	1.15:1	1.53	26
	200	150	10	10.67	0.94:1	0.18	10.67
	300	150	0	0	0	0	0

Table 2 continued.

Instar	Doses ppm	Larvae used	Sex-ratio				Adult emergence (%)
			Male (%)	Female (%)	Ratio	*t*-values	
7th	Control	150	40.67	39.33	1.03:1	0.18	75.33
	25	150	29.33	42	0.70:1	1.87	60.67
	50	150	32	28.67	1.12:1	0.52	50
	100	150	27.33	24.67	1.11:1	0.45	42.67
	150	150	21.33	21.33	1:1	0	29.33
	200	150	12	14	0.86:1	0.48	16
	300	150	0	0	0	0	0
8th	Control	150	38	43.33	0.88:1	0.73	72.67
	25	150	36	36	1:1	0	64
	50	150	26.67	34	0.78:1	1.2	54.67
	100	150	28	26.67	1.05:1	0.22	44
	150	150	20.67	23.33	0.89:1	0.49	31.33
	200	150	14	10	1.4:1	1.01	12
	300	150	0	0	0	0	0

Larvae of *A. diaperinus* showed tolerance to Btk treatment with the increase of their age (Table 1). The LD_{50} values were calculated from 62.773 (1st instar larvae) to 137.926 (8th instar larvae) ppm.

Adult emergence of the larvae fed on treated food was reduced significantly ($P < 0.001$). Emergence was inhibited more in the treated younger larvae than in the treated older larvae (Table 2). The effect of Btk on adult emergence showed positive relation with the dose.

A. diaperinus larvae gained tolerance against Btk treatment with the increase of their age. Larvae of *Plodia interpunetella* (Marden and Harein, 1984) and *Cadra cautella* (Faruki, 1994) showed similar trend of tolerance to Btk. In the present experiment it was found that longer exposure to Btk resulted in higher larval mortality. The toxic effect was reduced in older larvae (from 4th larval instar).

Toxins of *B. thuringiensis* damage the gut lining, leading to gut paralysis, and larval mortality is resulted due to starvation (Angus, 1956). The negative impact of Btk on the digestive system of *P. interpunetella* resulted in slower growth and prolonged developmental times (Schesser and Bulla, 1979). Total inhibition of pupal and adult recoveries in *C. cautella* was observed when young larvae fed on Btk treated food at doses > 200 ppm (Faruki, 1994). In the present experiment *A. diaperinus* larvae feeding on 300 ppm Btk treated food failed to pupate.

The findings of the present experiments revealed that longer exposure of *A. diaperinus* larvae to Btk treated food would be toxic and could reduce the population size by controlling pupation and adult emergence.

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(Received in May 2001; revised and accepted in April 2002)

ENTOMON 27(4): 447–453 (2002)
Short Communication No. ent.27417

Scanning electron microscopic studies on the post embryonic development of the dorsal eye of *Cloeon* sp. (Ephemeroptera: Baetidae)

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ABSTRACT: The post embryonic developmental changes of the dorsal eye of *Cloeon* sp. male in different stages are described. It has been observed that with the transition from aquatic to terrestrial way of life the hexagonal larval eye transforms into round facets in the subimago and finally to square facets in the imago contributing to increased resolution and scotopic vision in adults. The behavioural implications of the altered ommatidial structure in changed environmental light conditions have also been discussed.

KEYWORDS: *Cloeon* sp., ommatidia, imago, subimago

Mayflies (Ephemeroptera) are excellent material for investigations on the effects of aquatic pollution and ecological inter-relationships (Cummins, 1973; Merritt *et al.*, 1984). By comparison with other insects mayflies show a number of peculiarities and one of them is the presence of two large dorsal eyes in males, in addition to the lateral eyes (Zimmer, 1897; Streble, 1960; Meyer-Rochow, 1971; Horridge and McLean, 1978; Burghause, 1981; Horridge *et al.*, 1982).

Although numerous authors have studied optical and physiological characteristics of dorsal eyes of mayflies (Wolburg-Buchholz, 1976; Burghause, 1981; Meyer-Rochow, 1981), till now no information is available on the changes occurring during postembryonic development of the dorsal eye of mayfly. The present paper describes the morphological changes that occur in the dorsal eye of the larvae of *Cloeon* sp. belonging to the family Baetidae through different larval stages to subimago and imago and highlights the functional morphology of the dorsal eye of *Cloeon* sp. particularly in the subimago and imago stage as dorsal eyes play the most important role of recognising female for reproduction.

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Larvae of *Cloeon* sp. were collected from Ward Lake, Shillong (25°34′N; 90°52′E). They were sorted into developmental stages I–IV on the basis of wing pad development (Clifford, 1970). Male individuals were distinguished by the presence of dorsal eye and by their genitalia. Subimagines and imagines were obtained by rearing nymphs in well-aerated aquaria and in rearing boxes respectively. Because of the soft cuticle of subimagines and imagines specimens were fixed for 2–4 hrs in 25% glutaraldehyde buffered with 0.1 M Na-cacodylate. This was followed by washing in buffer, post fixation in 1% osmium tetroxide, dehydration in graded concentrations of acetone, and drying by 'Critical Point Drying' apparatus (Gupta *et al.*, 2000). Severed heads were mounted on brass stubs and coated with gold in a 'fine coat ion sputter' JFC 1100. Observations were made in a SEM (JSM 35CF) operated at 15 KV. Photomicrographs of the dorsal eyes of 10 male *Cloeon* sp. at each developmental stage were taken. Total corneal surface areas of the eyes and facet sizes of different developmental stages were measured from the photomicrographs. Where it was impossible to count the total number of ommatidia from the micrographs, the average size of one facet was calculated from 10 randomly chosen facets; total corneal surface area of the whole eye was then divided by this value to give the facet count (Meyer-Rochow *et al.*, 1990). Statistical analysis like 'Student's *t*-test' were carried out to see whether the differences between various parameters were significant or not (Zar, 1974). Facet diameter was defined as the longest diagonal distance on the outer surface of a facet.

The dorsal eyes are first discernible in the form of clusters of minute cuticular folds forming a roughly hexagonal ring enclosing a stretch of relatively smooth cuticle in the stage 2 larvae. From stages 2 to 3 and 4 there is a significant increment of facet count and corneal surface area, facet size remaining the same. At stage 4, ommatidia take a distinct hexagonal shape (Fig. 1). After the larval transformation into the subimago, an entirely different ommatidial structure is noted. The size of the individual facets register a 5 to 6 fold increase from stage 4 larvae with a significant increase in the corneal surface area. Surprisingly there is a significant decrease in the total number of facets (Table 1). The subimaginal dorsal eye exhibits a unique pattern where completely round and flat ommatidia are found distributed uniformly in the corneal surface area having sculptured interommatidial spaces (Fig. 2). Though ommatidial size and number of ommatidia continue to have an increasing trend during imaginal moult, corneal surface area is significantly reduced forming a turban shaped structure (Table 1). Consequently, there is a substantial increase in facet size and facet number, although corneal surface area registers a decrease. Ommatidia at this stage are square, puffy and compact without interommatidial space (Fig. 3).

It has been noted that the major observed changes in the facet size, facet count and corneal surface area occur during the transformation of aquatic larvae to terrestrial subimago which generally takes place in the twilight hours. The subimagines being winged yet sexually immature, do not need to locate females or to seek food, and in this short transitory period, while waiting for the final moult, their lone purpose is to avoid the predators. The possession of arrays of minute cuticular protuberances or corneal nipples in the subimaginal and imaginal ommatidia, serve this purpose by reducing

FIGURE 1. Dorsal eye of stage 4 larva (magnified). Bar = 1 μm.

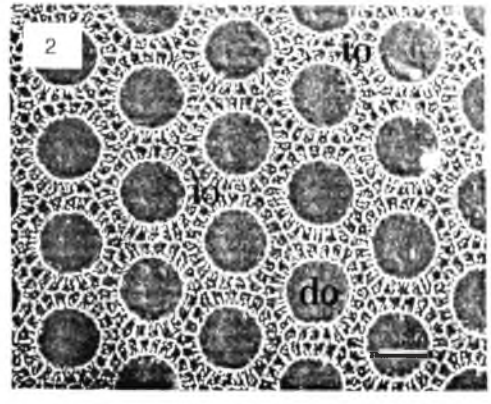

FIGURE 2. Dorsal eye of subimago (magnified). Bar = 10 μm.

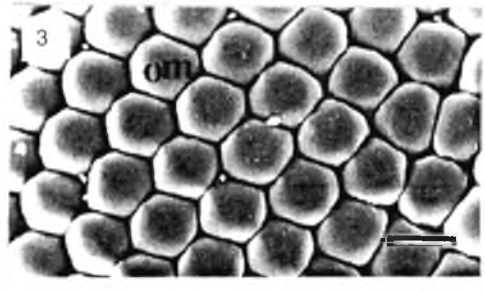

FIGURE 3. Dorsal eye of imago (magnified). Bar = 10 μm. **do** – developing ommatidium; **io** – inter ommatidial space; **om** – fully developed ommatidium.

TABLE 1. Comparison of mean ommatidial size, total number of ommatidia, and corneal surface area of the dorsal eye in nymphal stages 2 to 4, subimago and imago of male *Cloeon* sp. as revealed by 2 sample *t*-tests

	Size of ommatidia				Total number of ommatidia				Corneal surface area			
Aspects of comparison	*t*-test value	df	p	Result	*t*-test value	df	p	Result	t-test value	df	p	Result
Stage 2 vs. stage 3 nymph	0.6	8	NS	2 = 3	10.74	9	0.001	3 > 2	2.5	8	0.05	3 > 2
Stage 3 vs. stage 4 nymph	1.29	13	NS	3 = 4	23.12	9	0.001	4 > 3	13.49	9	0.001	4 > 3
Stage 4 nymph vs. subimago	16.83	13	0.001	5 > 4	76.93	8	0.001	4 > 5	6.6	9	0.001	5 > 4
Subimago vs. imago	3.844	8	0.01	6 > 5	3.09	9	0.02	6 > 5	6.54	9	0.001	5 > 6

Conventions: NS – not significant; P – probability; 2 – stage 2 nymph; 3 – stage 3 nymph; 4 – stage 4 nymph; 5 – subimago; 6 – imago

reflection and cutting off ghost images by increased efficiency of photon capture (Bernhard *et al.*, 1965, 1970; Gupta *et al.*, 1989). The significantly reduced corneal surface area of the turban-shaped eye may be explained by the fact that the interfacetal sculptured areas found in the subimaginal eye appear to have been incorporated into the imaginal facets. Hence, the visual field in the adult dorsal eye will decrease being placed on a cylindrical stalk in the one hand, and by a reduction in corneal surface area on the other. Sherk (1978) has clearly shown that the interommatidial angles would become narrower as the size of the visual field decreases and the number of ommatidia viewing this smaller visual field increases. It is also well known that the decrease in inter ommatidial angles brings about increasing capacity in resolution (Barlow, 1952; Sherk, 1977, 1978). Thus, the male *Cloeon* sp. by packing bigger sized, square shaped and increased number of ommatidia within a smaller corneal surface area achieve increased resolution, sensitivity and acuity (Barlow, 1952; Sherk, 1977, 1978). The huge eye with increased resolution sensitivity and acuity perform the main task of pursuing females during nuptial dance as males of the family Baetidae aggregate in swarms for performing non synchronised vertical dances and pursue anything flying overhead that vaguely resembles a female (Fischer, 1991). The reduced lateral eye are, however, expected to help the dancing males avoid collision between neighbouring dancing males by keeping a 'lateral eye' on each other (Gupta *et al.*, 2000). Furthermore, being sensitive to ultraviolet radiation (Meyer-Rochow, 1981; Horridge *et al.*, 1982), the males of *Cloeon* sp. which are twilight emergers could detect moving females against a background often rich in UV radiation but low in overall intensity (Gupta *et al.*, 2000). The transformation of the peculiarly rounded facets of subimago to square facets in the imago is important in the male mayflies, as square facets denote reflecting optics (Vogt, 1975; Land, 1976; Horridge and McLean, 1978) and are instrumental in scotopic i.e., dark vision. The significance of this transformation probably lies in the fact that like most aquatic insects mayflies are twilight emergers and the dorsal eye with its larger and square facets function as a dimlight receptor of high sensitivity. Since in this study transmission electron microscopy could not be done, we do not know about the presence of a clear zone between the optical array and the retina, which is the characteristic feature of the nocturnal insect (Land, 1981). However, the puffy appearance of the ommatidia may be the indication of the presence of haemolymph secreted during the transformation from subimago to imago separating the dioptric apparatus from the basal receptor so that Exner image falls upon the receptor layer (Streble, 1960; Meyer-Rochow, 1971; Wolburg-Buchholz, 1976).

Thus, the changes in dorsal eye morphology taking place during the subimaginal molt such as an increase in ommatidial size and number, decrease in interommatidial angles to improve resolution and a change from round to square facets to aid in scotopic vision together contribute to an improved ability in male imagines to detect the presence of females in a swarm and locate their position in order to facilitate mating. Hence it can be said that the evolution of the dorsal eye of *Cloeon* sp. appears to be linked with that of swarming behaviour of males during mating.

ACKNOWLEDGEMENT

We thank Dr. D. T. K. Khathing, Head, Regional Sophisticated Instrumentation Centre, North Eastern Hill University, Shillong for his kind help and encouragement.

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(Received in June 2001; revised and accepted in May 2002)

ENTOMON 27(4): 455–459 (2002)
Short Communication No. ent.27418

Longevity and fecundity of the uzi fly, *Exorista bombycis* (Louis) developed on different instars of silkworm, *Bombyx mori* L.

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ABSTRACT: Longevity, fecundity and size of the different stages of uzi fly, *Exorista bombycis* (Louis) developed on different instars of silkworm during different seasons of the year were investigated in the laboratory conditions. Results indicated that the uzi fly maggots did not develop completely on the first instar larvae of silkworm. The longevity and fecundity of the uzi fly were found high that developed on II to V instar larvae during rainy season compared to that of winter and summer seasons. Irrespective of seasons and instars, the longevity and fecundity were found highest in the uzi fly adults developed on V instar larvae.

KEYWORDS: longevity, fecundity, *Exorista bombycis*, instars

The tachinid fly, *Exorista bombycis* (Louis) commonly called uzi fly has been attacking the most economically important beneficial insect (silkworm) and hence called a pest. This uzi fly prefers to oviposite III to V instar larvae of mulberry silkworm resulting in serious damage on silk production (Krishnaswamy *et al.*, 1964; Jolly and Kumar, 1985). It is reported that the parasitization during the late or early V instar larvae is known to cause differential losses in terms of per cent shell weight and remarkable cocoon weight (Srikanth *et al.*, 1988). Accidental introduction of this tachinid fly into Karnataka from West Bengal, resulted in a serious threat to silk industry in south India. In the present investigation, as no information available in this aspect, the efforts were made to determine the longevity, fecundity and size of the different stages of uzi fly developed on different instars of silkworm larvae in the laboratory during different seasons as uzi fly parasitizes all the instars preferably III to V instar larvae in the field.

Silkworm larvae of different instars (I–V) were exposed to gravid females of uzi flies for oviposition in cages separately. The larvae of these instars were screened to determine the presence of uzi fly eggs. The silkworm larvae with 1–2 eggs on their body were selected for the present study. The silkworm of different instars with

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uzi eggs were reared separately till the emergence of the maggots. The maggots so emerged were collected and were allowed for pupation in the enamel trays containing a layer of sand. Emerged adult uzi flies from these pupae were collected and were provided with honey and sucrose solution mixture as diet. Each pair of these flies were provided with fifty V instar silkworm larvae for oviposition. The parasitised larvae were replaced by fresh ones daily till the death of the female uzi fly. Every day the total number of eggs laid by single female developed on different instars of silkworm were recorded till the completion of oviposition. The sizes of the male and female uzi fly adults developed on different instars were measured. The life span of these adults were also recorded. These experiments were conducted during different seasons of the year, hence the temperature and relative humidity recorded in the laboratory during summer, winter and rainy season were 30 ± 2.89 C, 55.85 ± 5.10%, 24.5 ± 1.90 C, 62.57 ± 4.80% and 21.55 ± 1.95 C, 76.25 ± 3.5% respectively.

The longevity and fecundity of uzi fly, *Exorista bombycis* developed on different instars of silkworm during different seasons of the year are presented in Table 1. When I instar larvae of silkworm were exposed to gravid female uzi files, no maggots were recovered though they were parasitised. No recovery of the maggots from this instar larvae may be due to insufficient food for completion of development and as a result the host and parasitoid are dead. Similar observations made by Santhosh *et al.* (1989) that even uzi fly infested II instar silkworm larvae died within 1 or 2 days due to their small size. The longevity and fecundity of *E. bombycis* developed on different instars were in increasing order from II to V instar (Table 1). Highest longevity and fecundity of uzi fly were recorded during rainy season than that of winter and summer. Irrespective of the seasons and instars, the longevity and fecundity of uzi fly were found highest in the adults developed on V instar larvae. It is evident that the longevity and fecundity of uzi fly determines the quantity of food material available during the feeding stage (maggot) of the uzi fly and congenial atmospheric conditions. According to Pradip Kumar *et al.* (1986) maggot recovery, pupal weight, fecundity of uzi fly decreased significantly when the density of maggots increased beyond five.

The size of the abdomen, thorax and wings of adult female and male uzi flies developed on different instars were measured and are presented in Table 2. It was found that the size of abdomen, thorax and wings of the female and male adults of uzi fly developed on II instar was least compared to later instars of silkworm. The size of the male and female adults developed on V instar of silkworm were measured and found highest (Table 2). It is evident from the result that the size, fecundity and duration of life of the different stages of uzi fly depends upon the availability of food from the host during feeding stage. The uzi fly infests all instars of silkworm larvae in the field. However, the loss due to this fly during the I and II instars is unnoticed by the sericulturists because of small size. It is suggested that the sericulturists should adopt the control measures from the first instar onwards till spinning to avoid crop loss and extra care should be taken from the IV instar onwards to curb the population build up.

TABLE 1. Longevity and fecundity of uzi fly *E. bombycis* developed from different instars of silkworm, *B. mori* L. during different seasons of the year

Seasons	Summer			Rainy			Winter		
	Life span in days			Life span in days			Life span in days		
Instars	Female (X ± S.E)	Male (X ± S.E)	Fecundity (X ± S.E)	Female (X ± S.E)	Male (X ± S.E)	Fecundity (X ± S.E)	Female (X ± S.E)	Male (X ± S.E)	Fecundity (X ± S.E)
II	4.77 ± 1.33	3.55 ± 1.12	149.10 ± 17.83	5.17 ± 0.15	4.03 ± 0.07	163.36 ± 3.86	5.58 ± 0.25	4.98 ± 0.08	172.00 ± 8.12
III	5.95 ± 1.05	4.89 ± 1.92	182.32 ± 19.85	7.29 ± 0.18	5.15 ± 0.38	180.27 ± 4.81	7.89 ± 0.81	6.15 ± 0.05	198.00 ± 5.61
IV	6.99 ± 1.06	7.22 ± 1.87	214.11 ± 37.60	9.69 ± 0.30	8.03 ± 0.29	287.25 ± 13.21	8.86 ± 0.98	7.80 ± 0.18	299.00 ± 6.90
V	9.44 ± 1.87	8.56 ± 0.88	221.22 ± 70.60	19.90 ± 0.62	9.05 ± 0.64	408.26 ± 31.15	14.56 ± 0.39	11.50 ± 0.59	454.0 ± 10.12
LSD (5%)	3.81 S		20.89 NS	1.69 S		8.64 S	1.69 S		1.70 S

TABLE 2. Size of male and female adults of uzi fly, *E. bombycis* (Louis) developed on different instars of silkworm, *B. mori L.*

Instars	Female						Male					
	Abdomen		Thorax		Wings		Abdomen		Thorax		Wings	
	L (cm)	B (cm)	L (cm)	B (cm)	L (cm)	B (cm)	L (cm)	B (cm)	L (cm)	B (cm)	L (cm)	B (cm)
II	0.43 ± 0.003	0.21 ± 0.003	0.29 ± 0.003	0.21 ± 0.00	0.54 ± 0.02	0.24 ± 0.003	0.45 ± 0.01	0.25 ± 0.00	0.30 ± 0.01	0.24 ± 0.00	0.62 ± 0.02	0.25 ± 0.01
III	0.48 ± 0.025	0.23 ± 0.01	0.31 ± 0.007	0.24 ± 0.007	0.69 ± 0.015	0.35 ± 0.01	0.54 ± 0.01	0.29 ± 0.006	0.37 ± 0.00	0.27 ± 0.007	0.75 ± 0.00	0.30 ± 0.01
IV	0.52 ± 0.003	0.28 ± 0.003	0.34 ± 0.003	0.29 ± 0.003	0.71 ± 0.025	0.38 ± 0.007	0.65 ± 0.02	0.32 ± 0.00	0.41 ± 0.007	0.30 ± 0.003	0.83 ± 0.01	0.32 ± 0.003
V	0.56 ± 0.007	0.30 ± 0.00	0.38 ± 0.007	0.31 ± 0.003	0.79 ± 0.01	0.44 ± 0.003	0.71 ± 0.01	0.34 ± 0.007	0.42 ± 0.003	0.31 ± 0.003	0.87 ± 0.001	0.38 ± 0.001

ACKNOWLEDGEMENT

The authors are grateful to the Director, and the Division Chief (Seri), KSSR&DI, Thalaghattapura, Bangalore-62 for facilities provided and encouragement.

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(Received in February 2001; revised and accepted in June 2002)

ENTOMON 27(4): 461–463 (2002)
Short Communication No. ent.27419

Efficacy of nuclear polyhedrosis virus formulations against *Helicoverpa armigera* (Hbn.) on sunflower

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ABSTRACT: Greenhouse experiment was conducted to know the efficacy of different NPV formulations @2.4×10^7 against 2nd instar *H. armigera* on sunflower. Among all the formulations, oil formulations @2.4×10^7 POB/ml were found to be significantly more effective followed by aqueous formulation @2.4×10^7 POB/ml, wettable powder formulations @6×10^8 POB/g and dust formulations @6×10^7 POB/g.

KEYWORDS: NPV formulations, *H. armigera*

INTRODUCTION

The head borer *Helicoverpa armigera* (Hbn.) a serious pest on several agricultural crops including sunflower. Control of this pest on different crops using nuclear polyhedrosis virus (NPV) in India has been suggested (Jayaraj and Rabindra, 1989). Earlier few attempts were made to produce suitable formulations of this virus (Ethiraju *et al.*, 1988; Rabindra *et al.*, 1991; Ignoffo *et al.*, 1991; Ignoffo and Gracia, 1994). This investigation deals with the evaluation of some NPV formulations against *H. armigera* larvae on sunflower heads.

The NPV was propagated in the fourth instar larvae of *H. armigera* and semi-purified by differential centrifugation. The polyhedral occlusion bodies (POB) was assessed using a neubauer haemocytometer and the virus was formulated into dusts, wettable powders and oil formulations. The dust formulations were prepared with chalk powder, Lignite, Talc and Flyash as carrier material, wettable powder formulations were prepared with Bentonite, talc and starch as carrier materials and oil formulations were prepared by mixing with neem oil and pundi oil (*Hibiscus cannabinus*).

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TABLE 1. Evaluation of different formulations of HaNPV on *H. armigera* under green house conditions

Formulation	Inert material used	Quantity of inert material used per 100 LE	Mean per cent mortality
Dust (concentration) @6 × 10^7 POB/g	Chalk powder	10 kg	16.66 (24.06)^h
	Lignite		25.00 (29.33)^g
	Talc		41.666 (40.18)^f
	Flyash		50.00 (44.98)^e
Wettable powder (6×10^8 POB/g)	Bentonite	1 kg	58.33 (49.81)^d
	Talc		41.66 (40.18)^f
	Starch		66.66 (54.72)^c
Oil (2.4×10^7 POB/ml)	Neem oil	250 ml	83.33 (65.98)^a
	Pundi oil	250 ml	75.00 (60.42)^b
Aqueous (2.4×10^7 POB/ml)	Water	250 ml	75.00 (60.21)^b
Untreated check	No spray	—	3.33 (10.50)^l
SEM±		**1.46**	
C.D at 5%		**1.46**	
C.V (%)		**5.77**	

Figures in parentheses are arcsine transformed values. In a column, means followed by the same alphabet do not differ significantly ($P = 0.05$) by DMRT.

To test the efficacy of formulations under natural conditions greenhouse experiment was conducted. This experiment comprised of 11 treatments and replicated thrice. Treatment detail are given in Table 1.

Recommended dose of fertilizer was applied to the pots before sowing. BSH-1 (sunflower var.) plants were grown in 33 pots for this experiment. When the plants were at 50 per cent flowering treatments were imposed. Dusting was done using a cloth bag with small pebbles inside. While dusting proper care was taken to avoid the problem of drift. While spraying was done with the help of hand sprayer and oil formulation was emulsified before spraying with wetting and spreading agent (Tritan x–100). After imposing the treatments laboratory reared second instar larvae were released two per head with the help of fine camel hair brush after careful checking and removing spiders and ants if any present on plants. The heads were covered with muslin cloth bag and observations were recorded daily on the mortality of larvae and it was expressed in percentage.

Results indicated that oil based formulations were superior over dusts and WP formulations, which were found superior over dust formulations and as good as aqueous formulation (Table 1). Among WP formulations starch based WP formulation was effective (66.66) followed by Bentonite WP formulation (58.33). Among dust

formulations fly ash based dust formulation was found effective (50.00) which was significantly superior over talc based WP formulation also. Talc based WP formulation and dust formulation were found equally effective. Chalk powder and lignite based dust formulations were inferior over all other treatments.

Increased efficacy of neem oil based formulation might be due to synergistic action. Dust formulations were found least effective compared to WP and oil formulations though flyash based (50.00%) dust formulation was more effective than talc based WP (41.0%) formulation. Ethiraju *et al.* (1988) also found WP formulations to be better than dust formulation of NPV against *H. armigera*. Similar findings were reported by Okada (1977). Thompson and Steinhaus (1950) also found the aqueous suspension of the polyhedra to be more effective for control of lepidopterous larvae than dust formulations. Better formulation technology and appropriate filler material may increase efficacy of dust formulation. Since the sunflower is grown primarily as a rainfed crop, the use of NPV dust formulations would be an appropriate technology in the management of *H. armigera*.

ACKNOWLEDGEMENTS

The financial help in the form of JRF from the Indian Council of Agricultural Research, New Delhi to the first author is greatfully acknowledged.

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(Received in September 2001; revised and accepted in March 2002)

ENTOMON gratefuly acknowledges the following reviewers, who offered their valuable time and expertise in evaluating the manuscripts during the year 2002.

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Edited, printed and published by D. Muraleedharan for and on behalf of Association for Advancement of Entomology, Department of Zoology, University of Kerala, Trivandrum. Typeset in TEX by River Valley Technologies, Trivandrum. Printed at St. Joseph's Press, Trivandrum